IS SEVERE VITAMIN D DEFICIENCY AN INDEPENDENT RISK FACTOR FOR CRITICAL LIMB ISCHEMIA(CLI) IN PATIENTS WITH PERIPHERAL ARTERIAL OCCLUSIVE DISEASE(PAOD)?

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT OF THE TAMIL NADU DR M.G.R. MEDICAL UNIVERSITY FOR THE DEGREE OF MCH VASCULAR SURGERY EXAMINATION TO BE HELD IN 2015

CERTIFICATE

This is to certify that "**Is Severe Vitamin D deficiency an independent risk factor for critical limb ischemia(CLI) in patients with peripheral arterial occlusive disease(PAOD)"** which is being submitted as thesis requirement for MCH vascular surgery examination of the Dr M.G.R Medical University of Tamil Nadu, is a bonafide work of the candidate – Dr. A. Dheepak Selvaraj.

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Dr. SunilAgarwal, Dr. Edwin Stephen, Dr. Prabhu Premkumar without whom this study would not have been possible.

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The Biochemistry Lab for their technical support.

CONTENTS

ABSTRACT

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Title: Is severe Vitamin D Deficiency an independent risk factor for critical limb ischemia of patients diagnosed to have peripheral arterial occlusive disease?

Hypothesis: Vitamin D is not a risk factor in peripheral arterial occlusive disease

Background: The role Vitamin D in metabolic homeostasis is well understood as it is required to maintain normal levels of calcium and phosphates. The Vitamin D receptors (VDR) are also present in the heart and blood vessels. In animal studies, absence of vitamin D receptors causes cardiovascular disease. Critical limb ischemia is a severe form of peripheral arterial occlusive disease (PAOD) which includes rest pain, tissue loss in the form of ulcer and gangrene. Medical therapy and surgical revascularization are the best options of treatment for limb salvage.

Methods: This was an observational crossectional study. Patients with atherosclerotic PAOD were classified into claudicants and critical limbs. Their serum Vitamin D levels were checked after informed consent. The results were analyzed using inferential statistics.

Results: There were 50 claudicants and 50 patients with critical limb ischemia. 80% were men and 20% were women. There was

statistically significant difference of severe vitamin D deficiency in the critical limbs compared to the claudicants (32.7%, 14% (p =0.004). Low ankle brachial index (<0.4) was related with severe vitamin D deficiency.

Conclusions: Severe vitamin D deficiency is an independent risk factor in critical limb ischemia.

LITERATURE REVIEW

INTRODUCTION

Peripheral arterial occlusive diseases are often not diagnosed and are the subject of suboptimal care. These patients have a higher risk of cardiovascular mortality when compared to age and sex matched control population. Hence the first line of treatment in these patients is always cardiovascular risk factor management with an aim of improving patient survival.

Patients with PAOD have highest rates of cardiovascular death, myocardial infarction and stroke when the patients are followed up for a year. (Figure 1) (1). The risk of a cardiovascular event in these patients seems to increase with the severity of the disease. So patients who present with rest pain or tissue loss seem to be at a higher risk compared to claudicants (Figure2) (2).

FIGURE1

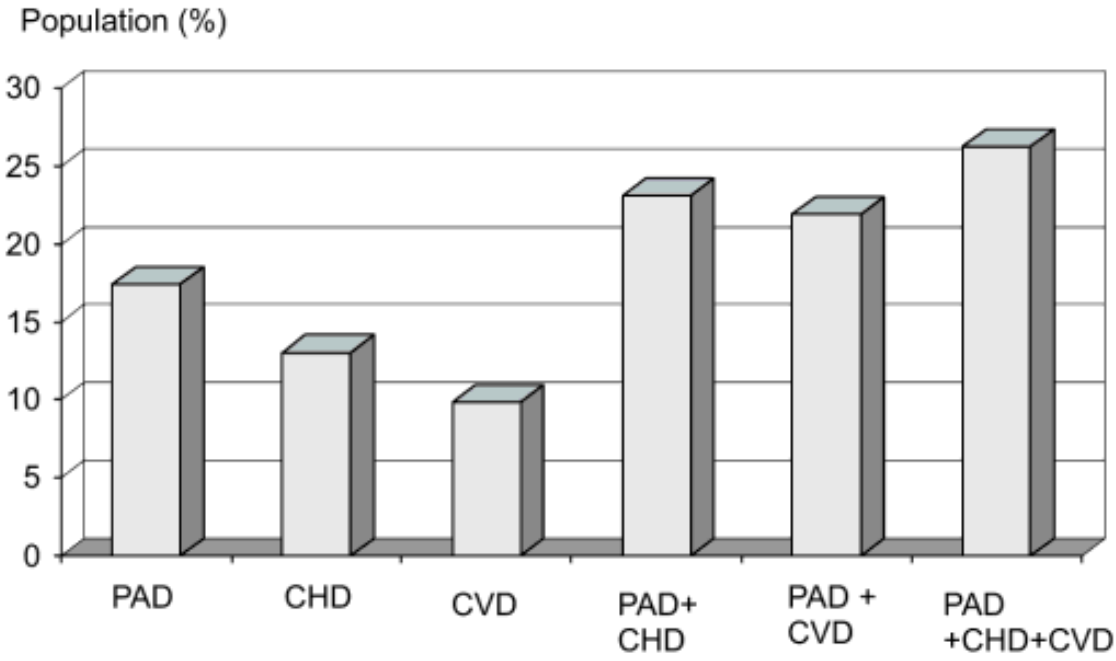
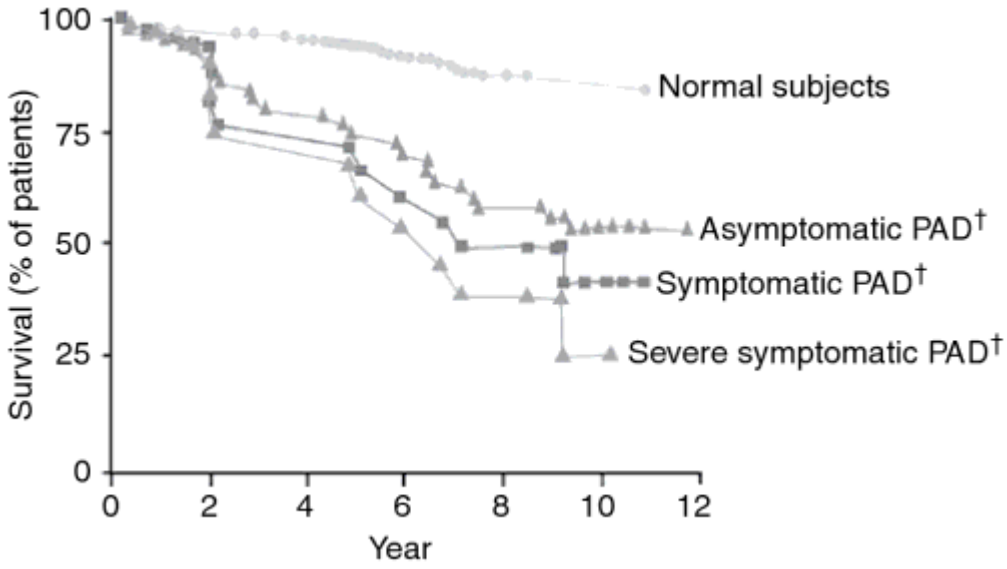

Population (%)
30
25
20
15
10
5
0
PAD
CHD
CVD
PAD+ CHD
PAD + CVD
PAD +CHD+CVD

FIGURE 2



Inspite of the increased rate of cardiovascular events seen in patients with PAOD, risk factor management in these patients has been woefully inadequate in our country due to various reasons. The general awareness in the community regarding the need for better control of hypertension, diabetes, lipids diet modification for these patients is not good. Public awareness of this condition is poor and thus the vascular surgeons have an important role in patient education as well as community awareness. These patients with PAOD received remarkably little attention in the primary and secondary health care set up in the treatment of these risk factors when compared to the coronary artery disease patients. In other words these patients received little or sometimes no treatment for lipid disorders or antiplatelet

therapy and most of these patients continue to smoke. Vascular surgeons need to not only treat patients but have to be involved in patient education as well.

The major risk factors for PAOD are the same as that for coronary arterial disease. These can be considered as modifiable and non-modifiable. The modifiable risk factors can be treated by lifestyle changes or diet and drug therapy. One of the important modifiable risk factor is smoking. Other risk factors include low HDL cholesterol levels, elevated LDL levels, high blood glucose and elevated blood pressure. There are several other factors like elevated homocysteine levels, prothrombotic factors, elevated lipoprotein levels, markers of infection that have also shown to be implicated.

Smoking cessation continues to be the most important modifiable risk factor. Smoking doubles the risk of disease progression. Smoking cessation definitely prevents or delays the onset of critical limb ischemia and also results in reduction of all major cardiovascular events.

Other risk factors like diabetes and hypertension have to be strongly controlled. All patients should be started on statins, antihypertensive therapy and good glycemic control.

There is also increasing data that a less vitamin D status may be an significant and ignored factor in the etiology of atherosclerosis and hence coronary and peripheral arterial diseases.(3,4)In general patients with PAOD, walk less due to claudication and rest pain followed by tissue loss. The exposure to sunlight seems to be less as compared to normal population. Vitamin D deficiency affects almost 50 % of the population worldwide. (3)The role of vitamin D in coronary artery disease and its deficiency as a risk factor has been the subject of recent studies. In our study we have compared the levels of vitamin D in two sets of comparable population (claudicants and those with critical limb ischemia) and have tried to answer our primary question. Is low levels of vitamin D a significant risk factor by itself in critical limb ischemia?. Future randomized control trials may be needed to see if actual vitamin D supplementation can delay the progression of the disease.

Vitamin D metabolism and mechanism of action

Majority of the vitamin D is derived from the skin following exposure to sunlight.D(.5) ultraviolet-B (UV-B) radiation causes the change of 7-dehydrocholesterol to previtamin D, which then trasforms to vitamin D.(6) Diet makes little contribution to vitamin D levels.(5) Vitamin D can be obtained from daily foods (e.g. UV-irradiated ,oily fish, eggs and dried mushrooms) or vitamin D sources.

The 2 major types of vitamin D are present: vitamin D3 and (cholecalciferol) and vitamin D2 (ergocalciferal). Cholecalciferol is obtained from production in the skin while Vitamin D2 (ergocalciferol) is derived from animal sources and diet.

Vitamin D as a compound exerts no important biological function. Two preliminary steps are needed to synthesize the

active vitamin D compound, 1,25-dihydroxyvitamin D [1,25(OH)2D].(6) The first step involves hydroxylation to 25(OH)D. This step occurs in the liver and is a substrate driven process. Then, the enzyme 1α-hydroxylase changes 25(OH)D to 1,25(OH)2D. The renal system is the important site for synthesis of functional 1,25(OH)2D. Blood levels of 1,25(OH)2D are hence determined by kidney 1α-hydroxylase activity, which is strictly regulated by factors linked to calcium and phosphorus metabolism [e.g. stimulation by parathyroid hormone (PTH) or the negative effect on fibroblast-growth factor 23].

Many peripheral tissues exhibit 1α-hydroxylase, which can produce important tissue levels of 1,25(OH)2D.(6) However the local availability of 1,25(OH)2D seems to depend mainly on the substrate availability of 25(OH)D. Moreover 24,25(OH)2D and 1,24,25(OH)3D are the main degradation products which is finally converted to inactive calcitroic acid which is soluble in water. Thus one theory of decreased availability of vitamin D especially in patients with CKD can be due to increased degradation.

Vitamin D metabolites bind to VDR's. This happens in the cytoplasm. After this the heterodimer complex translocates into the nucleus. In the nucleus the heterodimer complex reacts with certain DNA regions which are called vitamin D responsive elements. This substance controls 3% of the human genome.

VITAMIN D – REQUIRED DAILY ALLOWANCE

Due to avoidance of sunlight in the home bound population, elderly and use of sun protective factor have resulted in an increase of the dietary source of in the demand vitamin D. The RDA of vitamin D is 200 IU per day. In the absence of sunlight this is increased to two to three times the value. Vitamin D is present in many food sources both vegetable, animal and fortified food and milk. Early vitamin D deficiency is detected when the serum vitamin D levels drop below 20 mg/dl since at this level there is development of secondary hyperparathyroidism. Though home bound people are at high risk, studies have shown that the incidence even among the general population is high.

ASSAY OF VITAMIN D

Figure 3

DIASORIN- RADIOIMMUNOASSAY VITAMIN D ANALYSER

Serum concentration of 25(OH) D is good index of vitamin D level. . However, serum 25(OH) D levels do not reflect the amount of vitamin D accumulated in other body tissues. The blood levels 1,25(OH)2D is not an ideal

index of vitamin D status since it has a half-life of 15 hours and serum levels are closely linked to parathyroid hormone, phosphate and calcium(31). Levels of 1, 25(OH) 2D ideally do not decrease until vitamin D deficiency is very low..

Figure 4

Vitamin D analyzer

The levels of 25[OH] vitamin D correlate better with the clinical signs and symptoms because 25[OH] D is not tightly regulated and hence measurement of it more accurately reflects the body stores of vitamin D.

Figure 5

PTH ANALYSER

Schematic Diagram of Vitamin D Metabolism

Figure 6

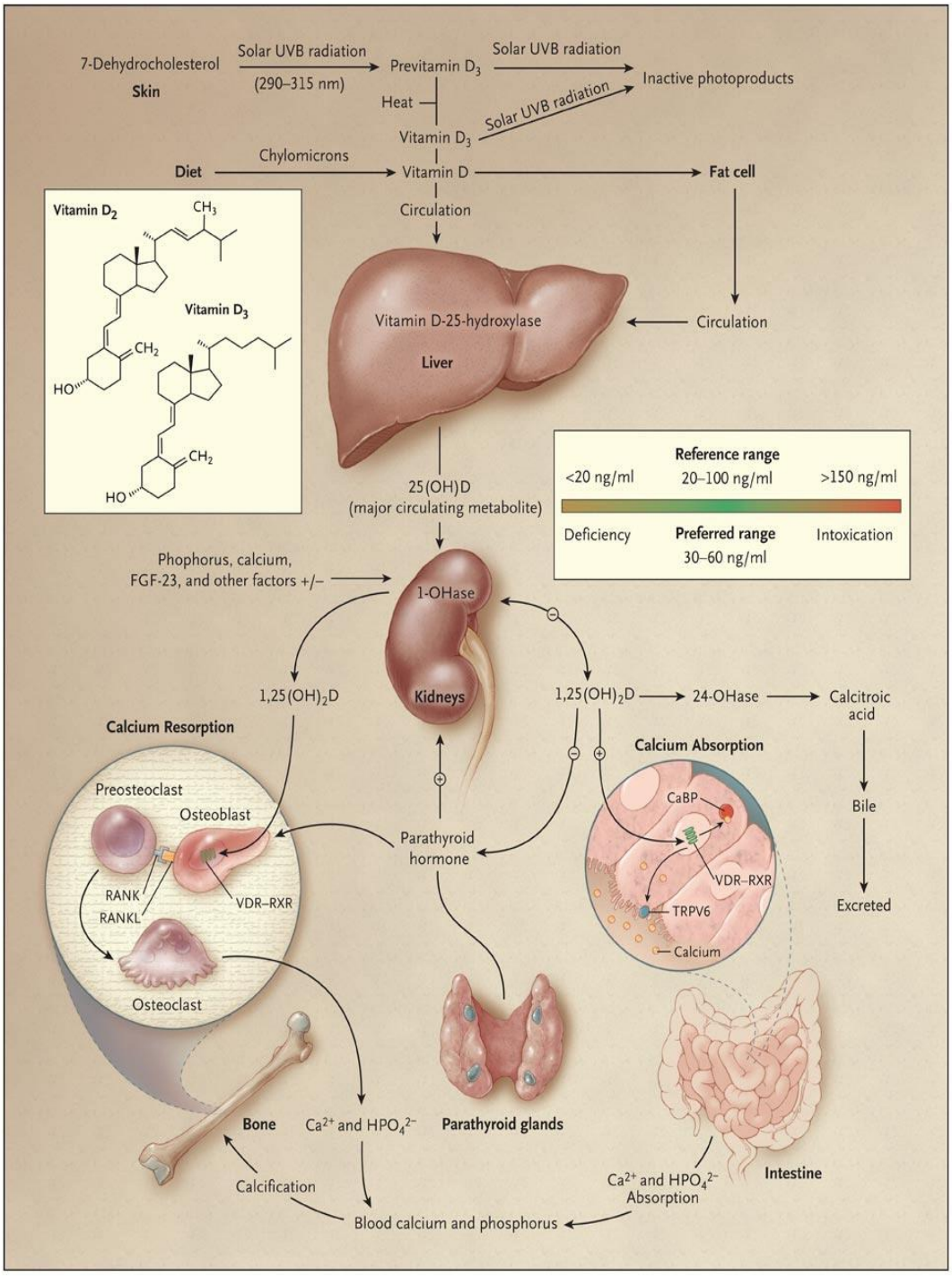
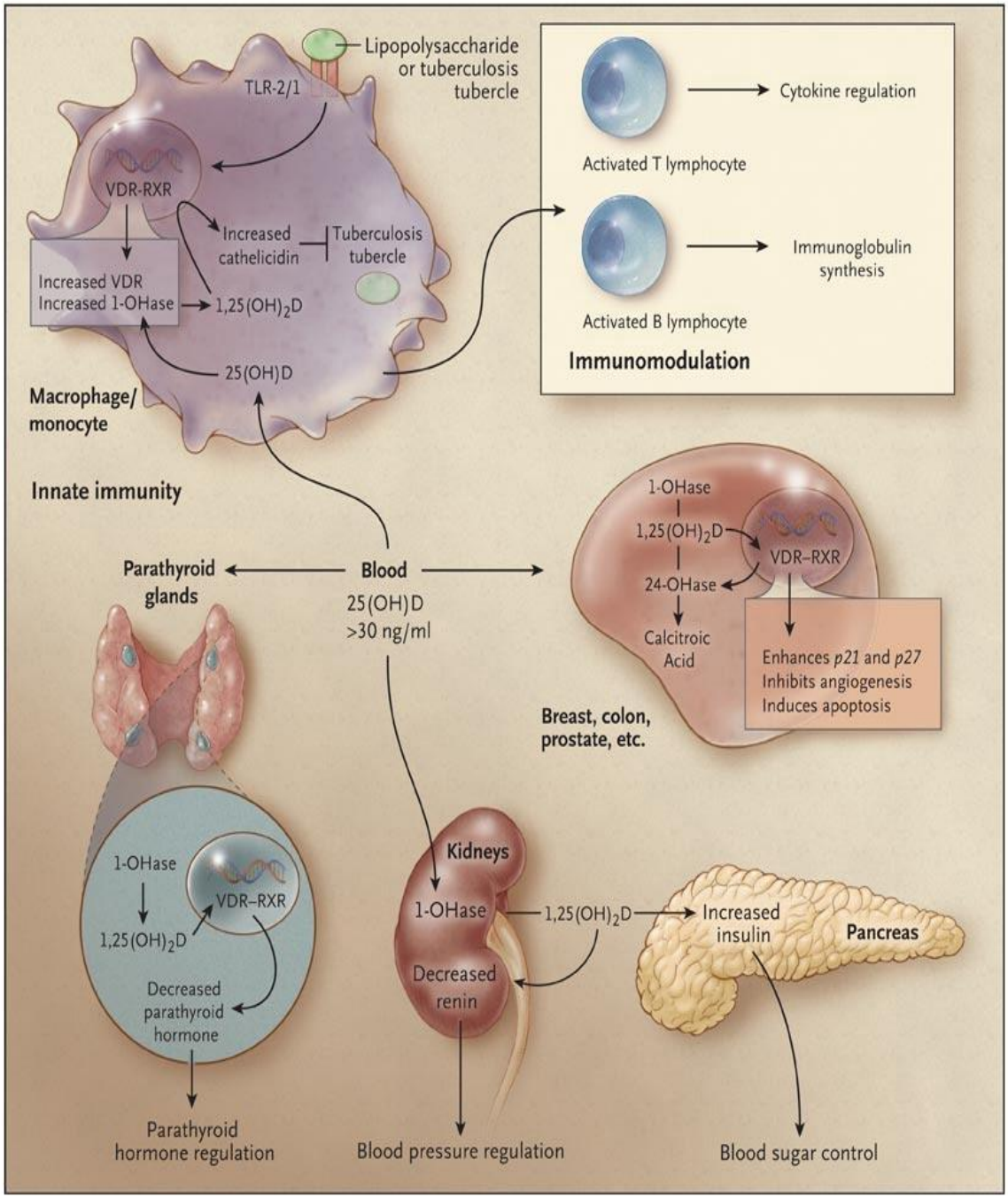


Figure 7


Lipopolysaccharide or tuberculosis tubercle
TLR-2/1
VDR-RXR
Increased cathelicidin
Tuberculosis tubercle
Increased VDR
Increased 1-OHase
1,25(OH)2D
25(OH)D
Macrophage/ monocyte
Innate immunity
Cytokine regulation
Activated T lymphocyte
Immunoglobulin synthesis
Activated B lymphocyte
Immunomodulation
Blood
25(OH)D
>30 ng/ml
Parathyroid glands
1-OHase
1,25(OH)2D
VDR–RXR
24-OHase
Calcitroic Acid
Enhances p21 and p27
Inhibits angiogenesis
Induces apoptosis
Breast, colon, prostate, etc.
Kidneys
1-OHase
1,25(OH)2D
Decreased renin
Increased insulin
Pancreas
Decreased parathyroid hormone
Parathyroid hormone regulation
Blood pressure regulation
Blood sugar control

The transformation of 25-Hydroxyvitamin D to 1,25-Dihydroxyvitamin D in the extra renal tissues is substrate driven and involves the Vitamin D receptor and stimulation of anti-inflammatory and immunogenic pathways

HISTORICAL PERSPECTIVE

Vitamin D was first named by McCollum et al in 1922 as the compound that can treat rickets.(7) The structure of 25(OH)D and 1,25(OH)2D was elucidated by 1930. In 1981 Robert Scragg (8) postulated that increased incidence of cardiovascular deaths in cold season may be due to less UV_B radiation and less Vitamin D levels. Vitamin D receptors were identified in the heart of rat by Robert Simpson in 1983 and this stimulated a lot of interest on the role of vitamin D in atherosclerotic vascular disease.(9)

Epidemiology of peripheral arterial occlusive disease

PAOD is very common in the community. Many patients are asymptomatic but lead a near normal life by altering their activities. They visit their physician when their disability becomes a handicap. Pain is always their first and foremost complaint. The Edinborough arterial study found that 4.5 % men and women over the age of 55 years had intermittent claudication, but a good 25% had asymptomatic disease. On follow up many new claudicants have come from the previously asymptomatic group. This study strongly indicates that there is window for altering the course of the disease by modifying the risk factors. However another important finding was that many of these patients do not actually deteriorate to the point of limb loss. In fact only 1 % - 2 % actually end up with an amputation. The rate of cardiovascular events among these patients is higher. If these patients are compared to normal individuals the rate of cardiovascular mortality is almost 6 times higher. 1 in 5 of the patients with critical limb ischemia will die within 1 year of diagnosis. These observations have resulted in a paradigm shift of treatment strategy which are now aimed at modifying risk factors.

CRITICAL LIMB ISCHEMIA :

The TASC II suggest that any patient with rest pain with or without ulcer or gangrene of more than 2 weeks duration with an ABPI of less than 0.5 or ankle pressure less than 50 have been defined as ctritical limb ischemia.

Measurement of ABPI :

Ankle brachial pressure Index is usually measured in the supine

position. The higher of the systolic blood pressure as measured in the dorsalis pedis artery and the posterior tibial artery(using a hand held Doppler) is divided by the higher of the brachial artery pressure in both the upper limb. The normal value ranges from 0.9 to 1.4. and values less than 0.9 are suspected to have an arterial pathology. Higher values of ABPI are seen in patients with calcific vessels as seen in longstanding diabetes and patients with renal failure.

Prevalence of vitamin D deficiency

The best method to study vitamin D status is Serum concentrations of 25(OH)D (10). A low vitamin D status leads to less intestinal calcium absorption and this will lead to less serum calcium levels. This will in turn stimulate PTH secretion. The ultimate result of high PTH levels on the gut [enhanced 1,25(OH)2D production], bones (calcium mobilization) and renal (e.g. reduced calcium loss) ensure enough physiologic blood levels of calcium levels. Depending on this understanding, the 25(OH)D level below which PTH levels begin to rise (approximately 75 nm; to convert 25[OH]D levels from ng/mL to nmol/L multiply by 2·496) is often used for the cutoff of a sufficient vitamin D level (10) Some physicians use less levels for vitamin D deficiency, such as cut-offs of <37·5 or <25 nm(11). A study in 207 rural and urban pregnant subjects in northern India showed there was significant vitamin D deficiency in both groups. 84 percent of women in both groups showed evidence of vitamin D levels below the cut off for normal(12). A study in school children in northern India showed the

same results with thirty six percent of children from low socioeconomic status showing a low serum vitamin D level(13). This problem does not seem to restrict itself to northern India as similar results were seen in a group of healthy south Indians . 44 percent of rural south Indians showed levels less than 10ng/ml in this study(14).

Mithal et al.(3) reviewed the overall vitamin D status less 25(OH)D levels (<75 nm) are highly common in all countries in the world(3) Advanced age, winter season, female sex, darker skin pigmentation, low intake of vitamin D and less sunlight exposure are some of the risk factors for vitamin D deficiency. Geographic latitude and altitude of residence have also been associated with cardiovascular mortality and morbidity. Seasonality of coronary arterial disease has also been observed. A study done in Australia identifies cardiac events were more likely to occur in colder season and spring than at rest of duration of the year,(15). Variation of UVB radiation and body levels of vitamin D with the seasons of the year may be responsible for this. Urbanization is associated with increased mortality from ischemic heart disease. In India especially risk factors for coronary disease is seen more in urban communities. This can be attributed to lifestyle, busy schedules of work, stress, unusual sleep, dietary habits and living in air-conditioned homes

with little exposure to sunlight. Pollution leads to excessive intake of UVB photons and thus reducing the creation of vitamin D in skin. Children who live in the outer regions Delhi have less levels of 25(OH)D concentrations in comparison with those living in lesser polluted areas.

For the first time when Hodgkin and colleagues published on the lesser incidence of vitamin D deficient rickets / osteomalacia in patients living in Punjab in India, no significant data appeared studying the vitamin D status of patients who live in tropical and sub tropical regions of the India till 1995.

The prevalence of vitamin D varies between latitudes and seasons. The incidence varies between countries widely since supplementation is done routinely in certain countries. In certain parts of the world where vitamin D added foodstuffs are available (USA and some Scandinavian countries), prevalence of vitamin D deficiency is between 1.6–14.8% in different age groups. In other European countries where there is no vitamin D supplementation, deficiency is more prevalent. The studies which assessed middle-

aged and elderly people showed vitamin D deficiency prevalence of 14% to 59.6% in these age groups. Vitamin D deficiency prevalence is much higher in Asian countries.

POPULATION AT HIGH RISK FOR VITAMIN D DEFICIENCY

1. Older adults

Americans who are 50 and older are at higher chance of getting affected with vitamin D deficiency. As population age, skin losses its ability to create vitamin D and the kidney loses its ability to change vitamin D to its metabolic hormone structure.

2. Limited sun exposure

Population living mostly at home, population who live in northern

latitudes (such as Alaska and New England), women who attire with long dresses with coverings for their head for religious implications and population with occupations that preclude them from sun exposure are less likely to obtain enough vitamin D from sun as the source.

3. Skin colour

Higher quantity of the skin pigment melanin will result in more colour to the skin and decrease the skin's capacity to synthesize vitamin D from sun exposure.

4. Fat metabolism.

As a vitamin which is fat soluble, vitamin D needs some fat in the food for absorption. Patients who have a decreased abilty to

intake dietary fat need vitamin D supplements. Fat malabsorption is linked with a vast number medical conditions like pancreatic enzyme deficiency, cystic fibrosis, Crohn's disease, surgical removal of part of the stomach or intestines celiac disease and some forms of liver disease.

5. Obesity

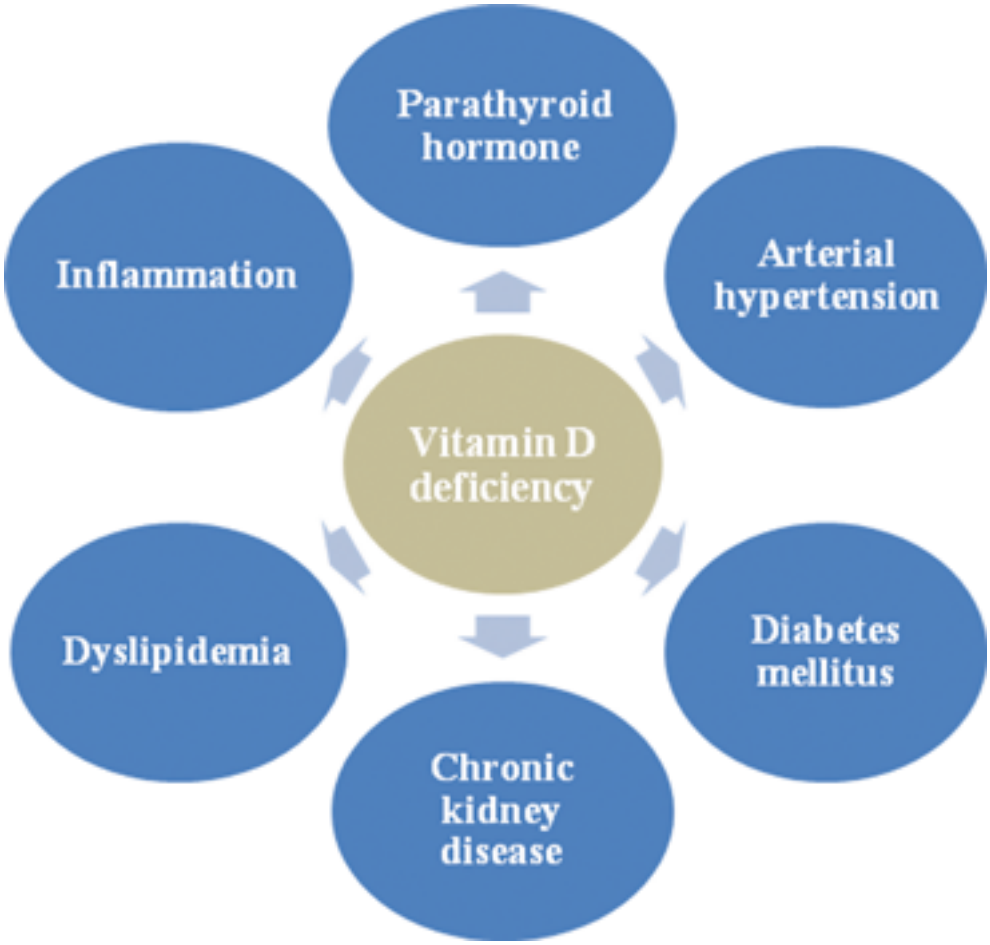
Patients with a body mass index (BMI) ≥30 mostly have a lesser plasma concentration of 25(OH) D, this level further declines as obesity and fat in the body becomes more . Obesity does not influence the ability of the skin to create vitamin D, but more quantity of fat in the subcutaneous plane trap more of the vitamin.

We should take into consideration the nature of 25(OH)D testing when assessing vitamin D status. (16) Whereas studies on standard

levels for vitamin D status are usually derived from Dia Sorin radioimmunoassay, the higher demand for 25(OH)D assays has evolved the creating of newer methods of analysis.(16)

There has been no general consensus regarding the cut off values for vitamin D deficiency across the world and RCT's are needed for standardization. We have noticed that the levels are generally lower in India inspite of the abundant sunshine.

Vitamin D and risk factors for cardiac diseases.



The relationship between vitamin D deficiency with cardiovascular risk factors:

Elevated levels of PTH are associated with increased cardiovascular morbidity. Vitamin D deficiency can lead to this kind of scenario.(17) PTH elevates the blood pressure and

influence through various pathways the heart including pro-arrhythmic actions and myocardial hypertrophy .(18).

Less levels 25(OH) D levels is an risk factor by itself for the incidence and prevalence of high blood pressure.(19) Moreover, meta-analyses of various randomized controlled trials indicate that vitamin D addition in different forms decreases the systolic blood pressure by 2–6 mmHg. (20–22)Vitamin D exerts its antihypertensive effects through Renal and vasculo-protective properties, PTH suppression and anti-inflammatory and antidiuretic actions. In addition, 1,25(OH)2D has been shown to overpower the transcription of renin (23). Studies in mice without VDR- and 1α-hydroxylasereceptors, display higher levels of the renin-angiotensin-aldosterone system (RAAS).(23)

Atherosclerosis is considered an chronic inflammatory disease. Previously the etiology of atherosclerosis was considered as a response to injury. Many scientist proposed that the destruction of endothelial cells is the first step of atherosclerosis. However nowadays people believe that endothelium which is surrounded by a milieu of cardiovascular risk factors like high levels of lipoprotein (LDL) result in loss of function and subsequently activating inflammatory

pathways.

This results in decreased production of nitric oxide and increased permeability of the endothelium resulting in entry of leucocytes and lipoproteins into the subendothelial space. This also results in the recruitment of smooth muscles cells into the space. This lipoprotein along with the smooth muscle cells in the subendothelial space are consumed by the macrophages to form foam cells. These foam cells are the loci of the lipid core resulting of the formation of the atheroma. Thus atherosclerosis has been recognized as an inflammatory disorder. The anti-inflammatory property of the vitamin d has been studied to influence the inflammatory response at the cellular level.

Atherosclerosis develops usually at specific site and this is usually are the bifurcation of major vessels. More good controlled studies are required to assess whether vitamin D levels are high at these endothelial cells specifically. Moreover recent studies have shown that metabolism of vitamin D is substrate driven.

The chronic kidney disease population are the most prone to

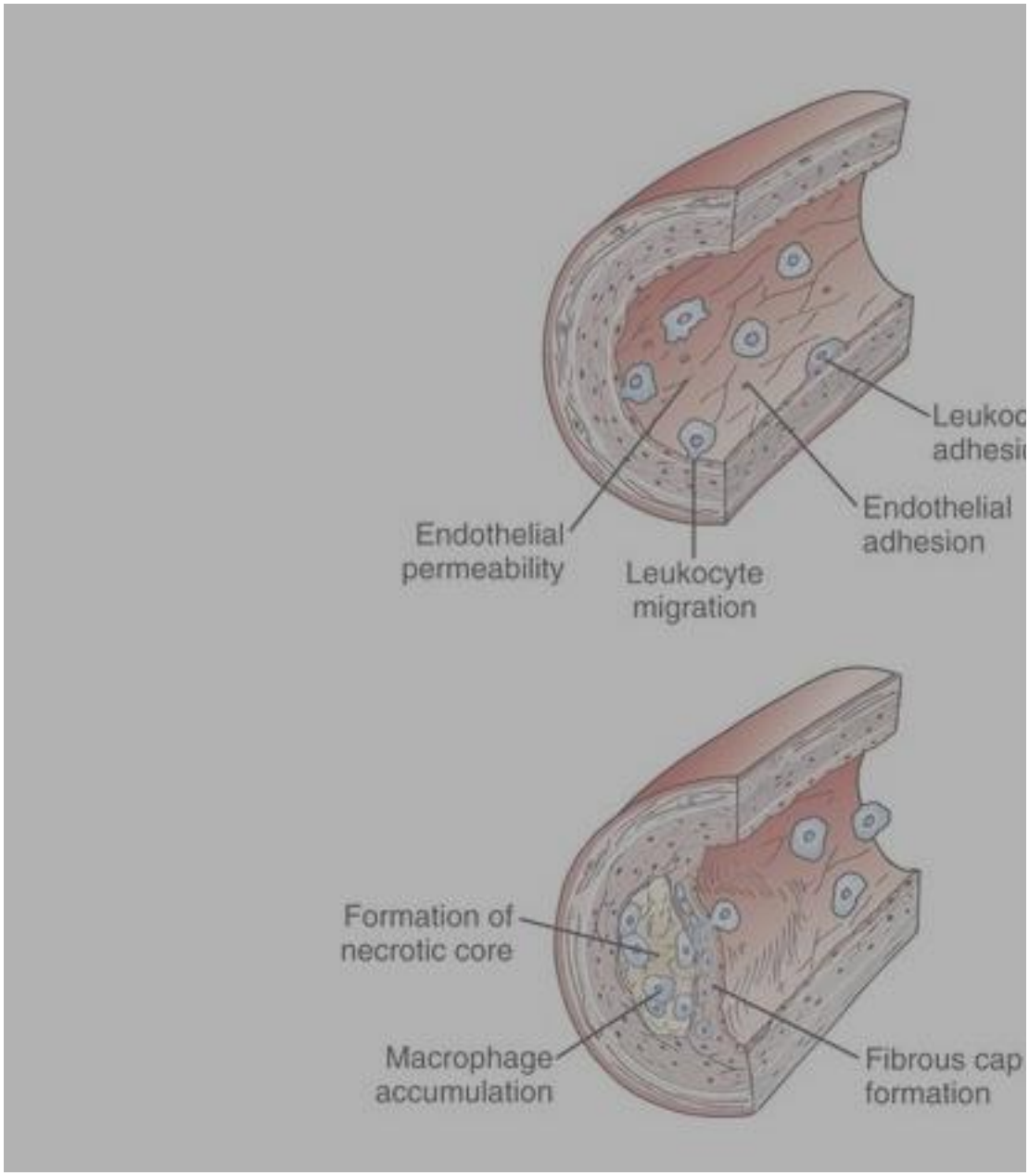
lesser levels of 25(OH)D levels.(24**)** These patents spend less time in the sun, have dietary restrictions and also show proteinuria which leads to urinary loss of protein-bound vitamin D compound (24) peculiarly, less vitamin D status is a independent etiology for decrease in functions of the kidney.(24)

Low levels of vitamin D status is in most scenario consistently, associated with a higher incidence and prevalence type 2 diabetes mellitus.(25) The active form of vitamin D stimulate insulin secretion and enhance insulin sensitivity.(25) Some, interventional studies have shown that glucose metabolism can be improved by vitamin D supplementation which reduces insulin resistance.(25) Vitamin D also has pivotal function in decreasing the prevalence type 1 diabetes mellitus. Less consumption of vitamin D in childhood and decreased solar UV-B radiation are linked with an higher chance of becoming type 1 diabetic.(25,26.27) Further RCT's are needed to prove if vitamin D supplementation can prevent diabetes.

Anti-inflammatory actions and anti-infectious actions of vitamin D such as decrease in the tumor necrosis factor-α (TNF-α) inflammation marker and elevation of the anti-inflammatory cytokine interleukin-10 (IL-10) are well studied

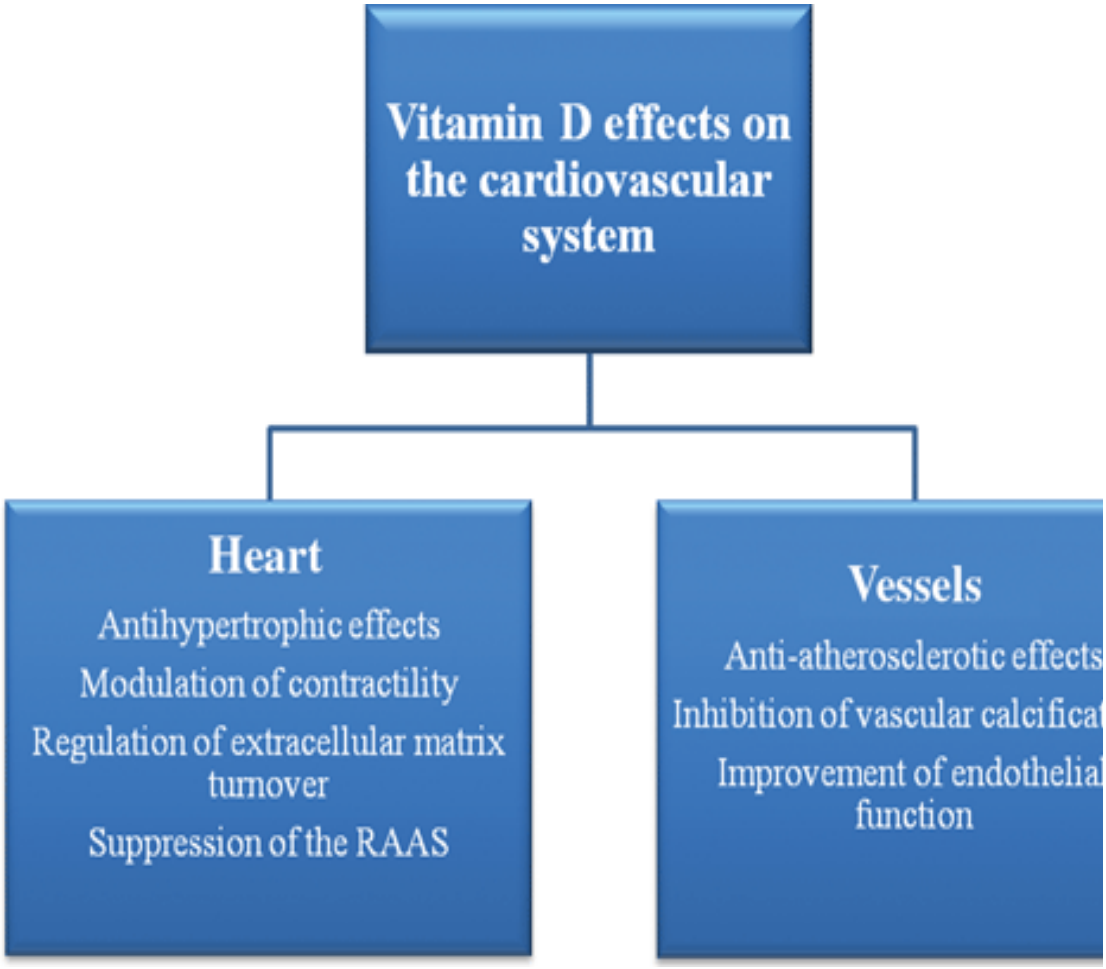
in the literature.(29–31)

Vitamin D contributes to other cardiovascular factors like coagulation parameters and blood lipids but the best data available to date cannot be used to finalize these conclusions (32,33)



Vitamin D effects on heart and blood vessels

Vitamin D exerts variety of effects on the heart and blood vessels (see Fig. 3). They are tissues at which the active form of vitamin D act upon and exhibit both1α-hydroxylase and VDR .(34–37)



Proposed vitamin D effects on blood vessels and heart :

1α-hydroxylase-knockout and Vitamin D receptor-knockout mice suffer from heart failure despite normal levels of calcium levels. **(**38,39) More activation of the RAAS can be ascertained as the mediating pathway since RAAS blockade

with, for example, the angiotensin-converting enzyme (ACE)-inhibitor captopril completely inverts cardiac abnormalities in these mouse models.(38,39). There is also higher VDR expression in myocardial hypertrophy. (35, 36, 36.) Various studies have shown anti- proliferative and anti- hypertrophic effects of vitamin D compounds, such as decreased regulation of genes which are active in the hypertrophy of cardiac muscle fibres. (40,41) VDR up regulation positively favors cardiac calcium channels and promotes relaxation of cardiomyocytes, thus improving diastolic function of the heart.(42) Cardiac extracellular matrix turnover which is under the influence of Vitamin D-mediated regulation may also be important in the maintenance of cardiac health.(45,43) A analysis of 171 British Bangladeshi adults showed that MMP-9 is higher in vitamin D deficient patients but is less after vitamin D supplementation.(43) Patients with cardiac failure generally have low vitamin D levels but the significance of the relationship needs further evidence

Vitamin D may also have a protective effect against vascular calcification, atherosclerosis, and endothelial dysfunction.(44) The main antiatherogenic effects of vitamin D is mediated through VDR. This is present in endothelial cells and vascular smooth muscle cells. EC's or endothelial cells play an

important role by up regulating NO and antioxidant effect. Moreover vitamin D also has an anti-inflammatory and anti antigenic effect. Other effects include down regulation of cholesterol uptake by the macrophages and foam cell proliferation and decreased in vascular smooth muscle cell activation and migration.(44–46.47). Finally, vitamin D decreased calcification of the vessel , for example, by down regulating bone morphogenic proteins, but data on this issue is contentious.(48). This could be ascertained by the data that low levels of vitamin D in the blood and vitamin D overdose may be a risk factor for vascular calcification, though it noted that the largest data on vitamin D and calcium augmentation in the food did not reveal any effect on coronary artery calcification.(49)

Based on the data from various study population it is evident that vitamin D insufficiency is associated with an high risk of cardiovascular morbidity and mortality(50–53). In the study of very specific cardiovascular outcomes, a relevant relationship between decreased 25(OH) D and strokes which resulted in decreased mortality was revealed. Grandiet al.(53) studied that the cardiovascular morbidity was higher by 83% (hazard ratio 1·83; 95% CI: 1·19–2·80) in patients with decreased 25(OH)D levels, that is, population with 25(OH)D

levels less than or ranging from approximately 25 to 50 nm. Data of RCTs studied mainly to correlate effects of vitamin D augmentation on cardiovascular diseases are still not clear. Further RCTs are underway to definitively study the relationship between vitamin D supplementation and cardiovascular morbidity in the different population, but the results and outcomes to be published and to be relevant for our clinical practice is a long way ahead (e.g. the VITAL; see http://www.vitalstudy.org).

PERIPHERAL ARTERIAL OCCLUSIVE DISEASE AND

VITAMIN D

From this line of thought it seems easy to assume that vitamin D levels in the serum might have an association with peripheral arterial occlusive disease. Low vitamin D is more common in adults with type 2 diabetic and is independently related with higher carotid intima-media thickness (54).While this is not definite evidence of PAOD this does impress upon us, the effect vitamin D levels have on atherosclerotic disease. A paper published in 2012 on the effect of nutrition and composition in the body in PAOD studied that decreased synthesis of vitamin D is related to deterioration of symptoms of PAOD.(55)

We have already stated that vitamin D insufficiency acts as an independent etiological factor for atherosclerosis though it acts on vascular smooth muscle cell activation, endothelial cell dysfunction and lipid peroxidation. A study on vitamin D deficiency and impaired bone turn over with low bone mass in patients with PAOD demonstrated that in a group of patients with angiographically confirmed PAOD, 11% had mild vitamin D deficiency and a further 60% had severe vitamin D deficiency (56) A study published in the European Journal of

vascular and Endovascular surgery in 2011 showed that vitamin D was associated with more aortic stiffness and calcification of the peripheral arteries(57). This was followed by another study from the Erasmus university Rotterdam, Netherlands that said Vitamin D might actually be an independent risk factor for arterial disease (58).

The prevalence of vitamin D deficiency in the Indian population is quite significant with range from 50 %to 90% over all age groups and populations. Independent cohorts studied in our hospital have shown a 20% prevalence of severe vitamin D deficiency (<10nm) in the population. However data on the burden of vitamin D insufficiency and its relationship with PAOD is lacking.

METHODS

Aim and Objectives

Aim- To evaluate if severe vitamin D deficiency in patients with atherosclerotic peripheral arterial occlusive disease is a risk factor for critical limb ischemia

Objectives

(1) To test serum vitamin D levels for patients with atherosclerotic PAOD

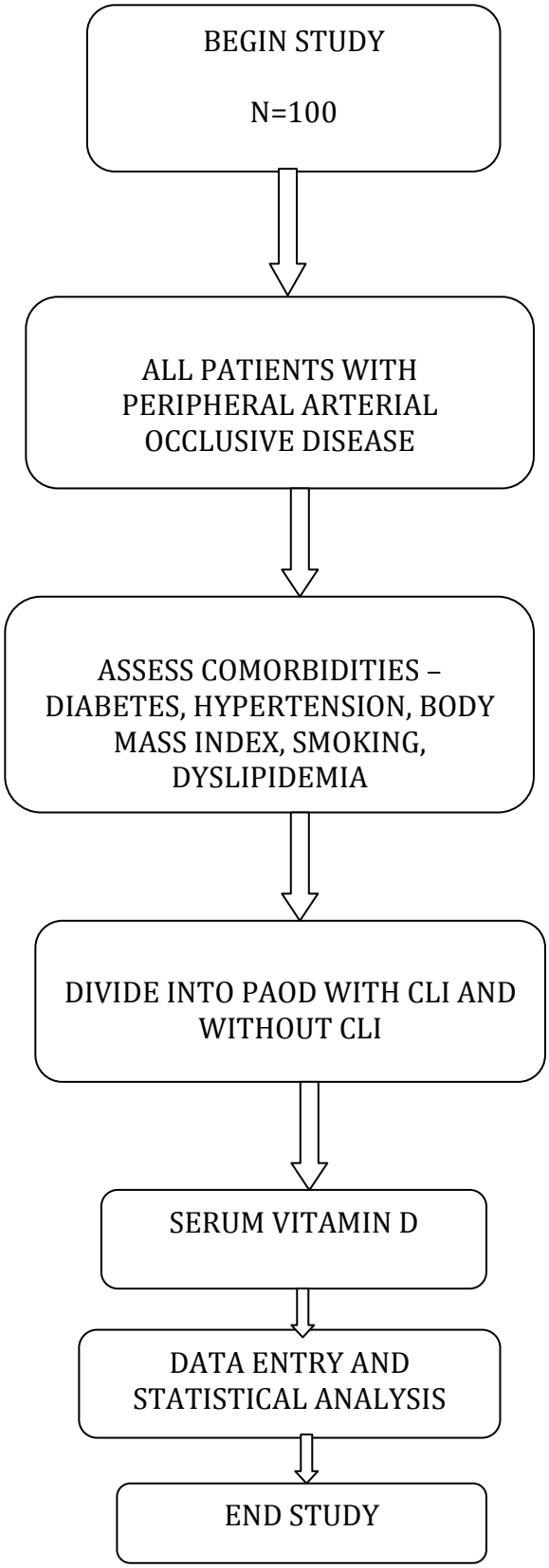
(2) To analyze if levels of vitamin D are lower in patients with critical limb ischemia as compared to claudicants.

STUDY POPULATION RECRUITMENT

This study is an observational cross sectional study. All patients who were diagnosed to have PAOD were divided into two groups. One group of claudicants and another with severe disease (critical limb ischemia). Critical limb ischemia is defined as history of rest pain; tissue loss in the form of ulceration or gangrene with ABPI of less than 0.4. ABPI between .9 and 1.4 is normal. Any ABPI between .4 and .9 is subcritical. All these patients underwent a thorough history and examination followed by Ankle-brachial pressure index evaluation. The ABPI was measured at resting with a portable 8MHz vascular Doppler. The ABPI was derived by dividing the higher of the right and left systolic ankle pressure (posterior tibial or dorsalis pedis) by the higher of systolic blood pressure in the brachial artery, according to the Trans Atlantic Inter-society consensus management guidelines (TASC) Serum vitamin D was then tested by radioimmunoassay. Severe Vitamin D deficiency was defined as a serum level of less than 10mg/ml.

Diabetes(fasting plasma glucose>120mg/dl;2 hour post prandial plasma glucose > 200mg/dl, hypertension(blood pressure of more than 140mmHg systolic and 90mm of Hg diastolic), dyslipidemia (total cholesterol>240mg/dl), smoking history was also noted

Detailed diagrammatic algorithm of the study:


BEGIN STUDY
N=100
ALL PATIENTS WITH PERIPHERAL ARTERIAL OCCLUSIVE DISEASE
ASSESS COMORBIDITIES – DIABETES, HYPERTENSION, BODY MASS INDEX, SMOKING, DYSLIPIDEMIA
DIVIDE INTO PAOD WITH CLI AND WITHOUT CLI
SERUM VITAMIN D
DATA ENTRY AND STATISTICAL ANALYSIS
END STUDY

Detailed research plan:

a. Setting:

This study will be carried out in the Vascular Surgery and Biochemistry department of the Christian Medical College, Vellore.

The study will be conducted over 18 months.

We will recruit 100 patients in 2 subsets of 50 each

Inclusion Criteria – Males

Age – 50 – 75 years of age

i. SUBSET I

PAOD without Critical Limb ischemia

a. Absent Pulses

b. ABPI <0.9

ii. SUBSET II

PAOD with Critical Limb Ischemia (any of the following)

a. Rest pain

b. Tissue Loss (Ulceration / Gangrene)

c. ABPI <0.4

Exclusion Criteria – ThrombangitisObliterans

Patients on Vitamin D Supplements

Metabolic bone disorders

Disorders of Fat Malabsorption

Setting – Vascular Surgery OPD

Sampling – Venipuncture for Vitamin D, Consecutive Patients

b. **Participants:**

As above

c. **Variables** –

Patients taking Vitamin D supplements

The above will be determined in the outpatient department by a standard format questionnaire with simple yes/no answers to minimize collection of aberrant data.

Potential confounders

Vitamin D supplementation prior to testing

d.**Data Sources / Measurement**

I. **Vitamin D** – Serum vitamin D was measured in fresh blood samples using a 25-hydroxyvitamin D radioimmunoassay (Diasorin Inc., Stillwater, MN, USA). At our institution any level less than 30ng per milliliter is considered deficient. Less than 10ng per milliliter is considered severe deficiency. Any level between 10 and 20 is considered moderate deficiency and a level between 20 and 30 is considered as mild deficiency. Any level more than this is generally considered adequate.(59,60)

f. **Sample Size:**

Study Basis for sample size calculation

Osteoporosis (2005) 16: 319-324 Hypovitaminosis D, impaired bone turnover and low bone mass are common in patients with peripheral arterial disease Astrid Fahrleitner – Pammer, Andrea Obernosteror, Ernst Pilger, Harald Dobnig, Hans Peter Dimai, Georg Loeb, Stefan Kudlacek, Barbara M.

"Patients with PAD IV showed significantly lower vitamin D levels than patients with PAD II. We found significantly higher PTH, AP and CTX levels and significantly lower serum calcium in PAD IV patients. Eleven percent of patients with PAD II, but some 59% of patients with PAD IV, had serum 25 (OH) D levels below 9mg/ml and were classified

as vitamin D deficient. Consequent secondary hyperparathyroidism was detected in 49% of PAD IV patients and in 11% of PAD II patients."

Though the above study shows a 60 percent prevalence of severe Vitamin D deficiency in critical limb ischemia along with 11 percent prevalence in Fontaine grade II, this is without considering Fontaine stage I as well and we expect, in the Indian population for this number to reach close to thirty percent. Hence keeping the two percentages at 30 and 60 we get the following sample size calculation.

Estimated sample size for two-sample comparison of proportions

Test Ho: p1=p2, where p1 is the proportion in population 1

And p2 is the proportion in population 2

Assumptions: Alpha = 0.0500 (two sided)

Power = 0.8000

P1 = 0.3000

P2=0.6000

N2/n1=1.00

Estimated required sample sizes: n1 =49

n2 = 49

g. Quantitative Variables:

Serum Vitamin D levels. Assessed by radioimmunoassay. The Vitamin D levels in the patients without critical limb ischemia will be compared to those with critical limb ischemia and analyzed.

h. Statistical Methods:

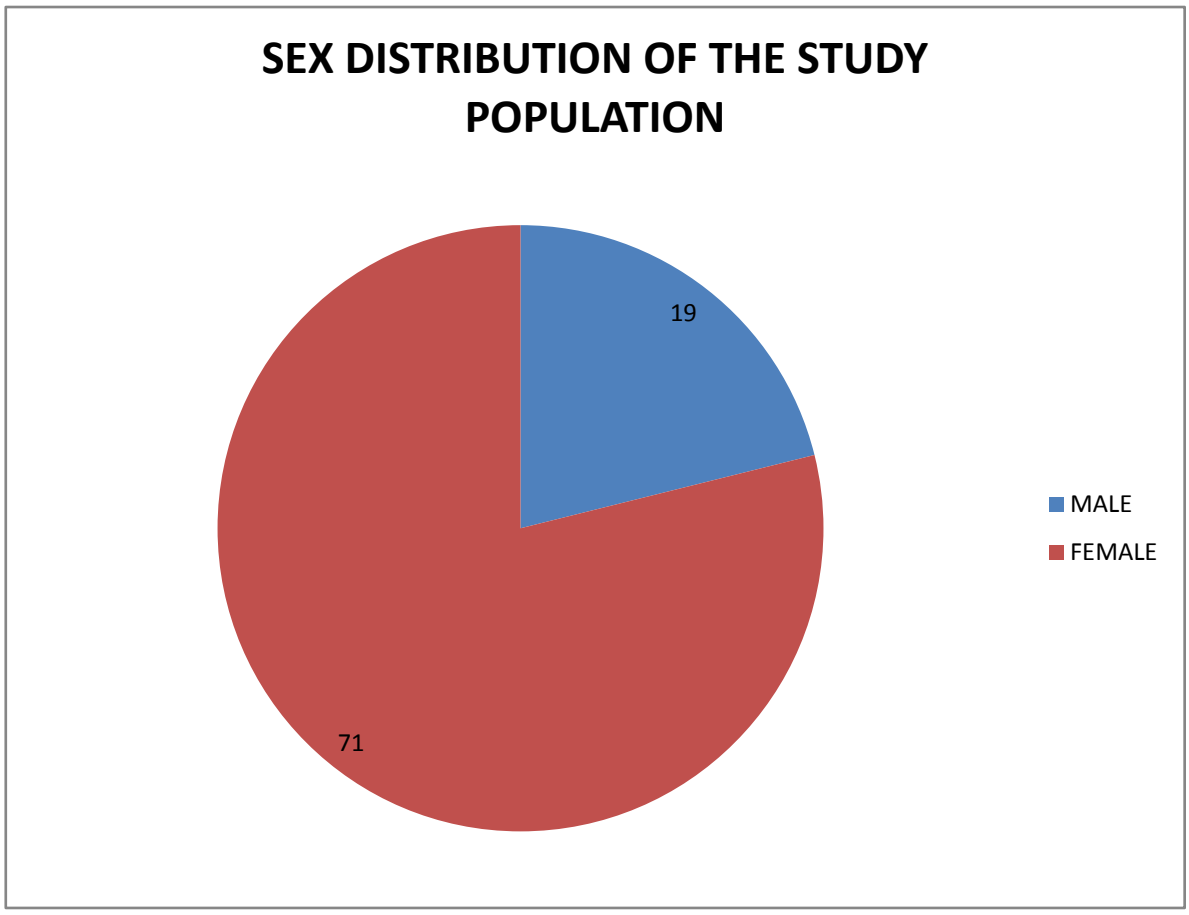
Chi Square test will be used on the two groups while we will be using the independent T test to analyze continuous variable. As all the tests will be done at the point of care. Missing data is not anticipated.

RESULTS

RESULTS

A total of 100 patients were included in the study. 50 patients had claudication and 50 had critical limb ischemia. All these patients were seen by a vascular surgeon in the outpatient clinic .A detailed history and examination was done. This was followed by an ABPI evaluation. The inclusion and exclusion criteria were strictly followed. There were 80 men and 20 women. The mean age of the population was 58.9 years. A total of 24 patients were severely deficient (36). 36 patients were moderately deficient and 23 patients had mild deficiency and 17 patients had normal values of vitamin D. Lower ABPI levels were associated with more severe deficiency .The other cardiovascular risk factors like diabetes, hypertension and hyperlipidemia showed a similar distribution between the two groups of patients.

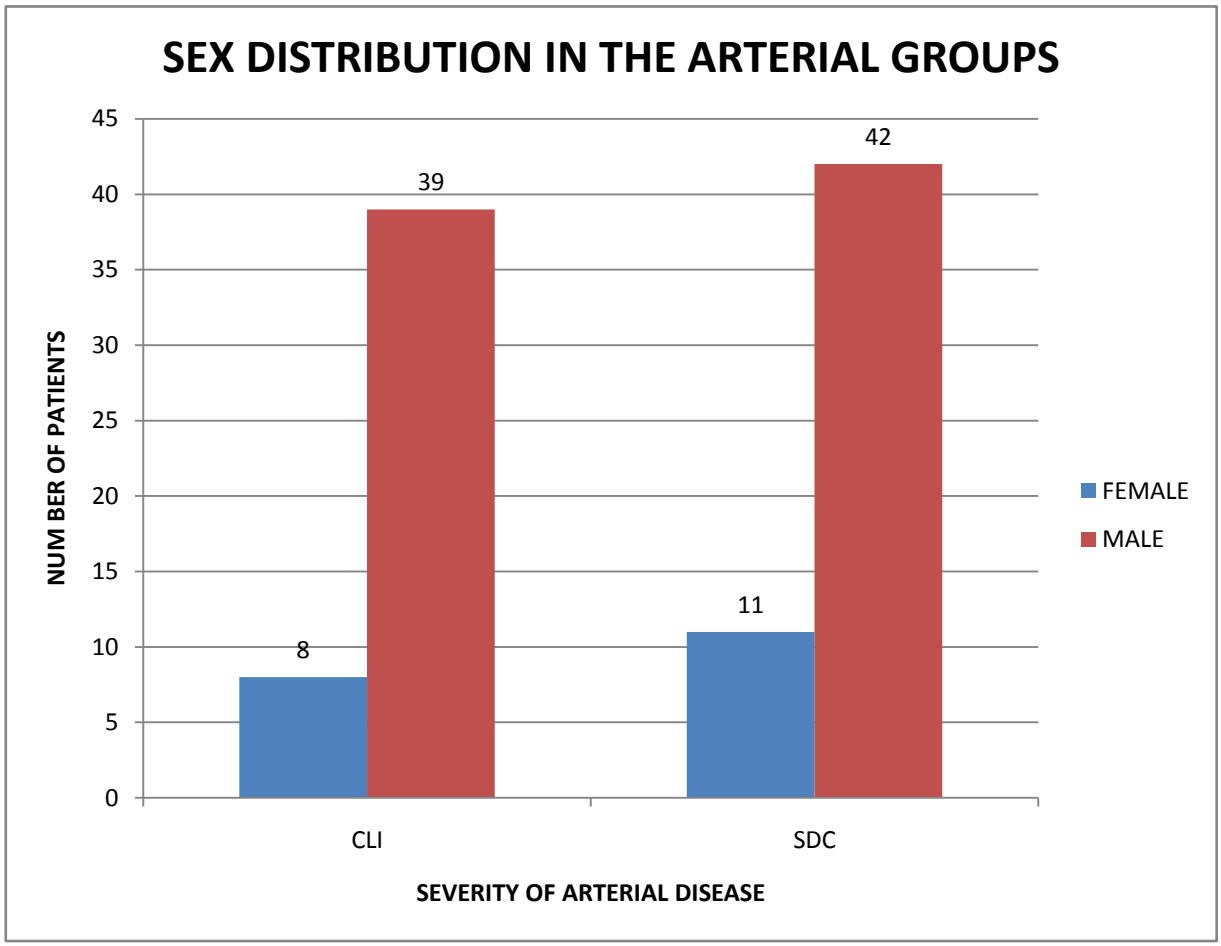
Graph1



Majority of our study population were male patients. There were 71 male patients

and 19 female patients.

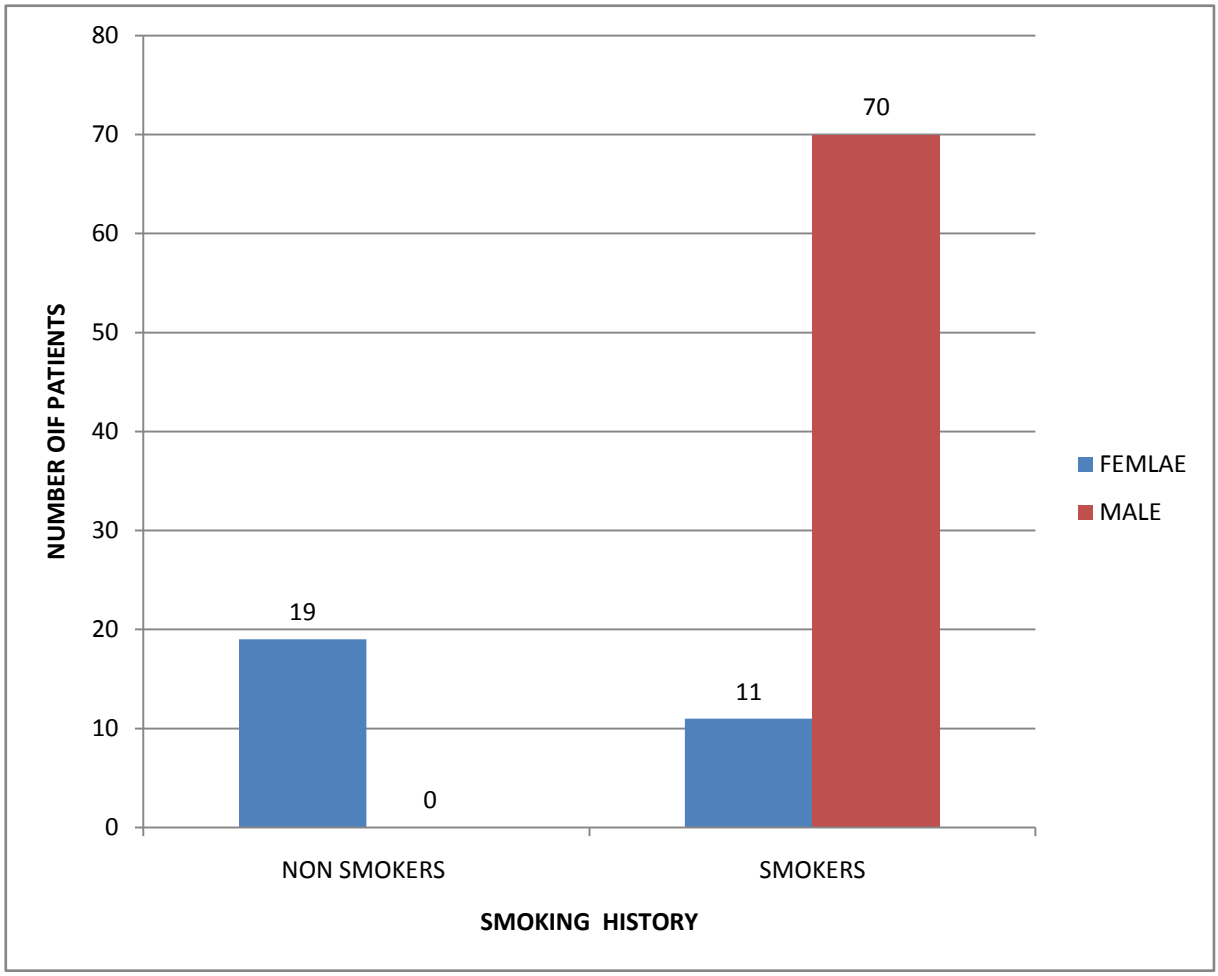
Graph2



There were 8 female patients in the critical limb iuschemia group and 11 patients

in the claudicant group. There were 39 male patients in the critical limb ischemia group and 42 male patients in the claudicant group

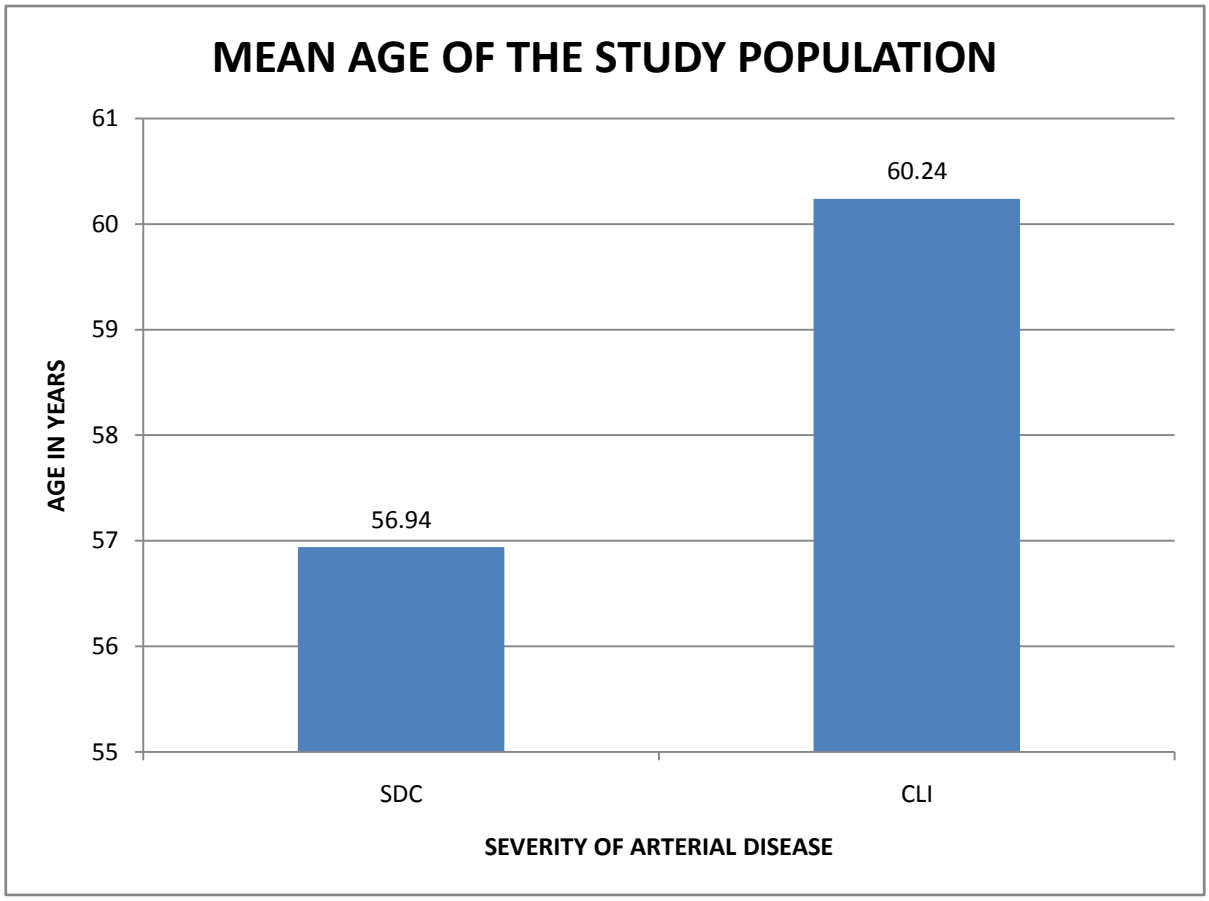
Graph 3



All the females were non smokers while 11 males were non smokers and the rest

of the male patients were smokers. This graph shows that smoking as a risk factor among female patients with arterial disease is not very common occurrence due to cultural difference as compared to the west but still may be seen in some states of India .

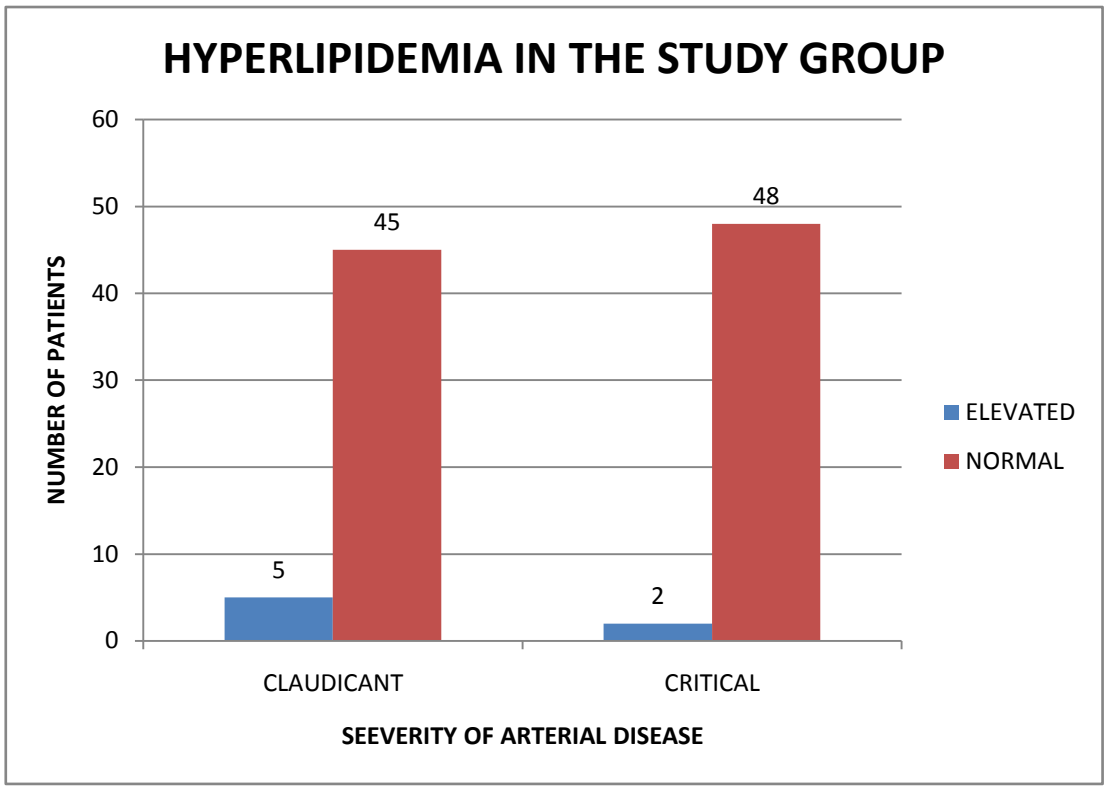
Graph 4



The mean age in the claudicant group was 56.94 years and the mean age of the

critical limb ischemia group is 60.24 years. Since age is a risk factor for atherosclerotic disease, the age distribution in both the study groups were not significantly different

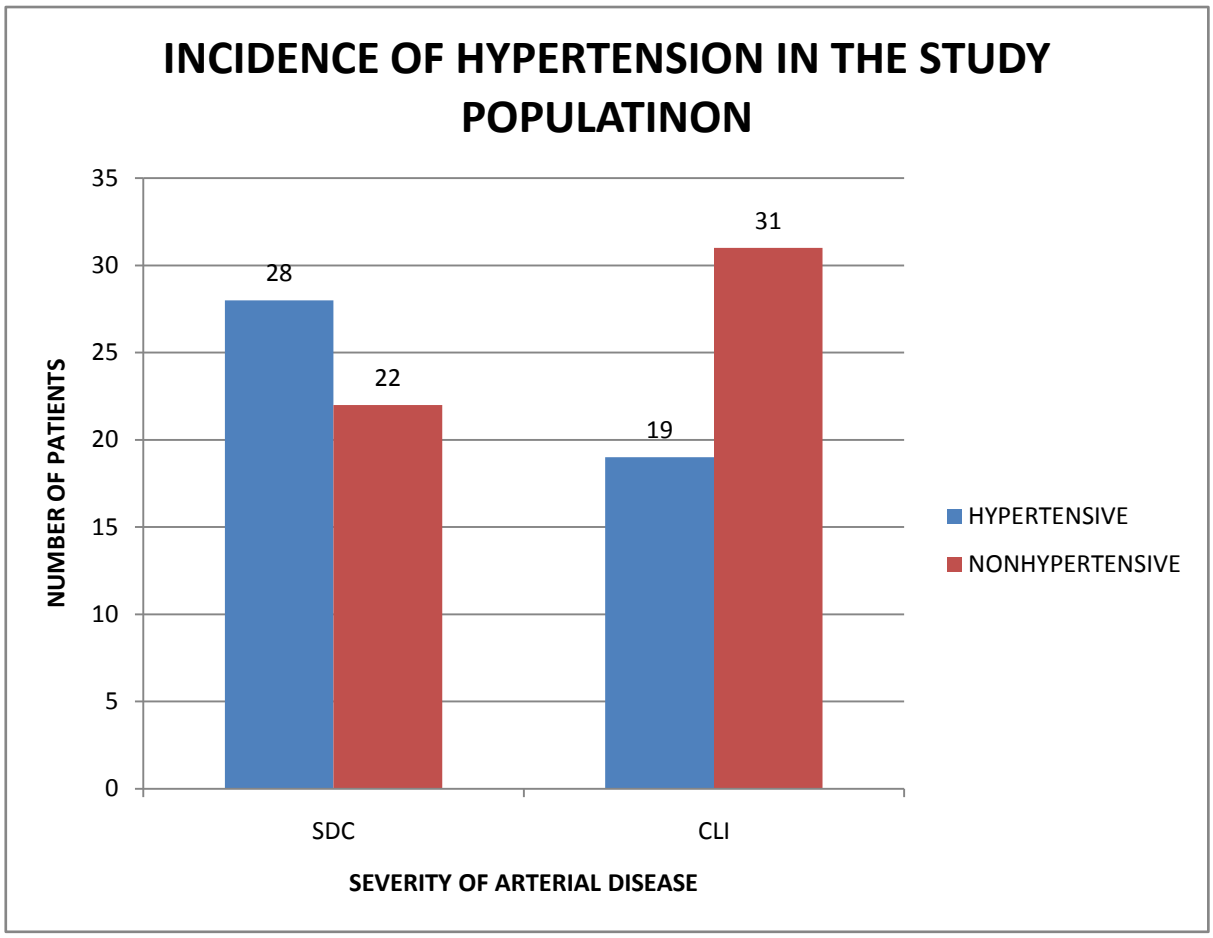
Graph 5



There were 5 patients in the claudicant group with hyperlipidemia and 2 patients in the critical group with hyperlipidemia. Most of the patients had normal levels

of serum cholestrol. This is in contrast to other western data where hyperlipidemia is a known risk factor in the progression of atherosclerotic disease.

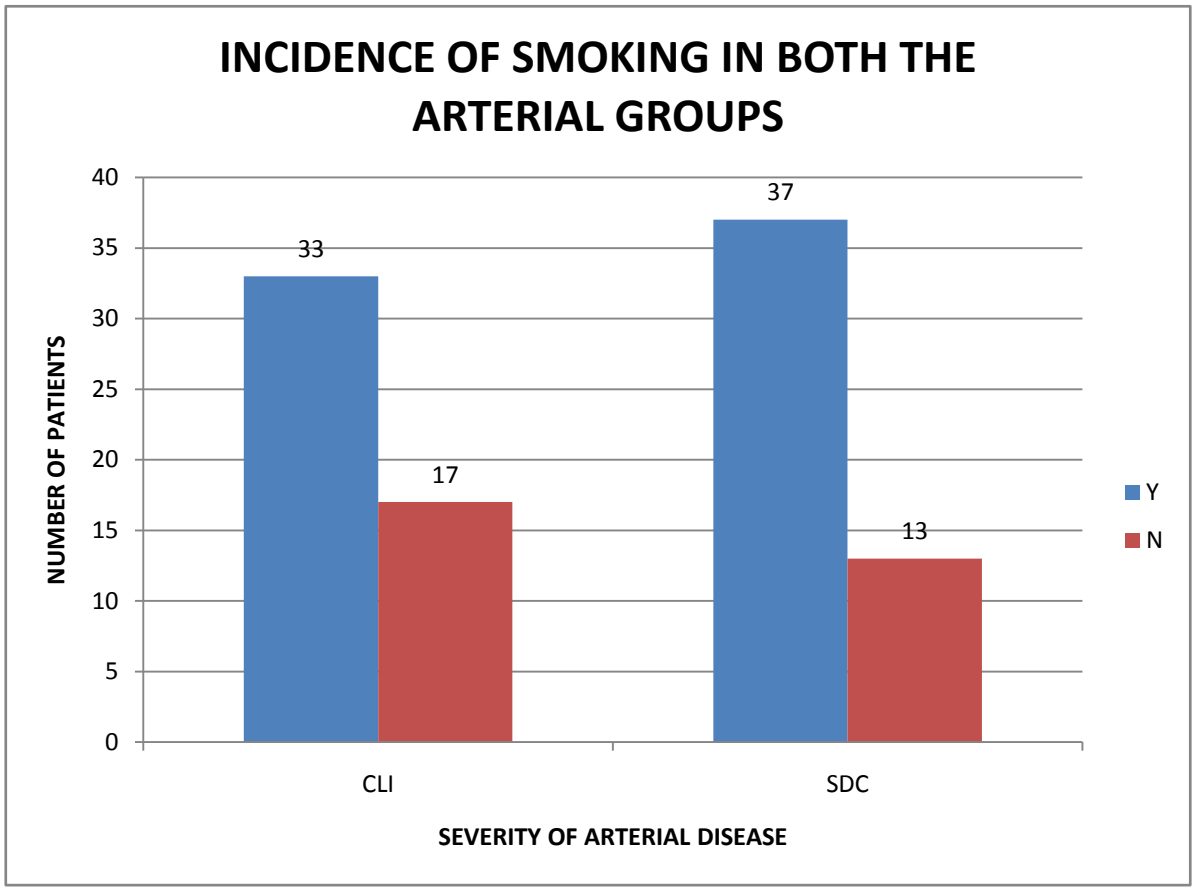
Graph 6



There were 28 patients in the claudicants group with hypertension and there were 19 patients in the critical limb ischemia group with hypertension. Comparing the

incidence in both the group it was not statistically significant.

Graph7



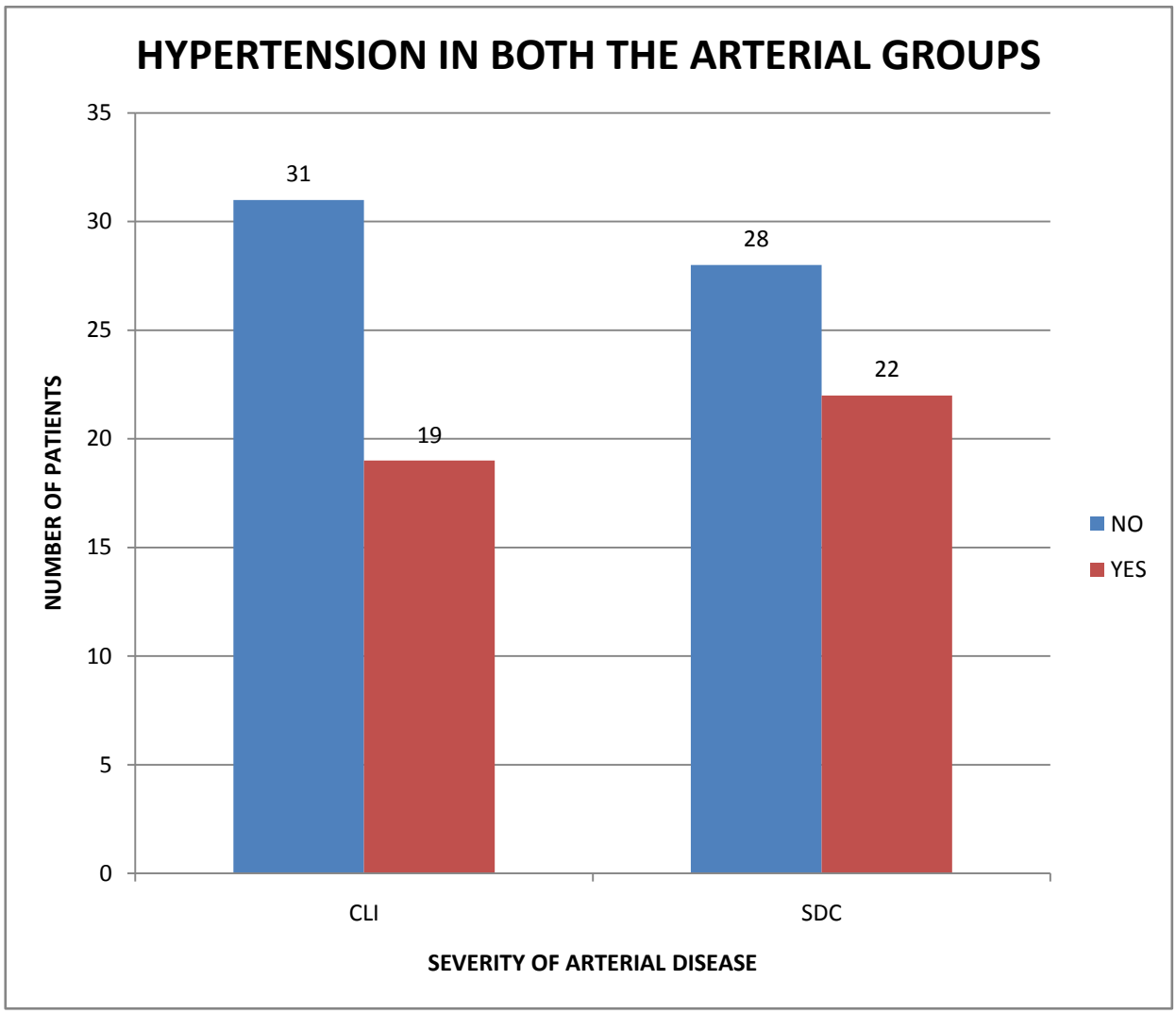
33 patients in the critical limb group were smokers (66 %) as compared to 37

patients in the claudicant group (74 %). The incidence of smoking as a risk for cardiovascular disease is similar in both the study groups.

Cardiovascular morbidities

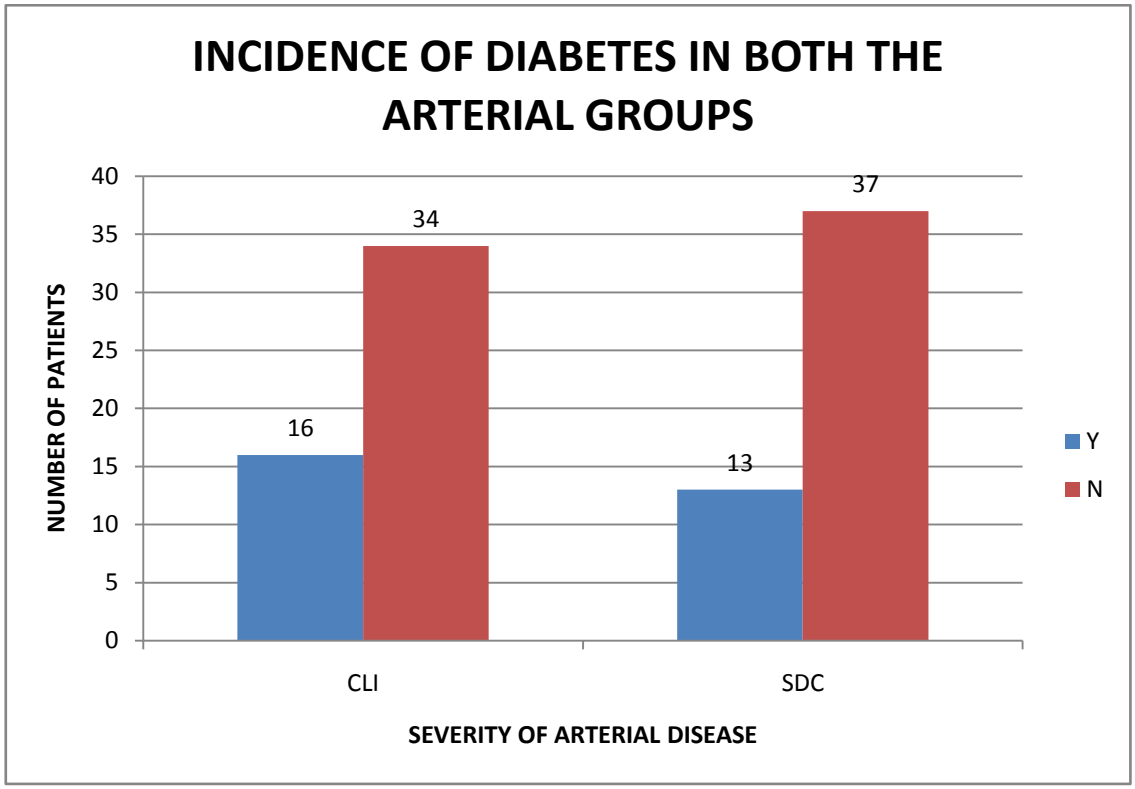
The risk factors analyzed were hypertension, diabetes mellitus and hyperlipidemia. Overall lower vitamin D levels seemed to be associated with more risk factors. There seemed to be more current smokers in the group with severe deficiency and critical limb ischemia.

Graph8



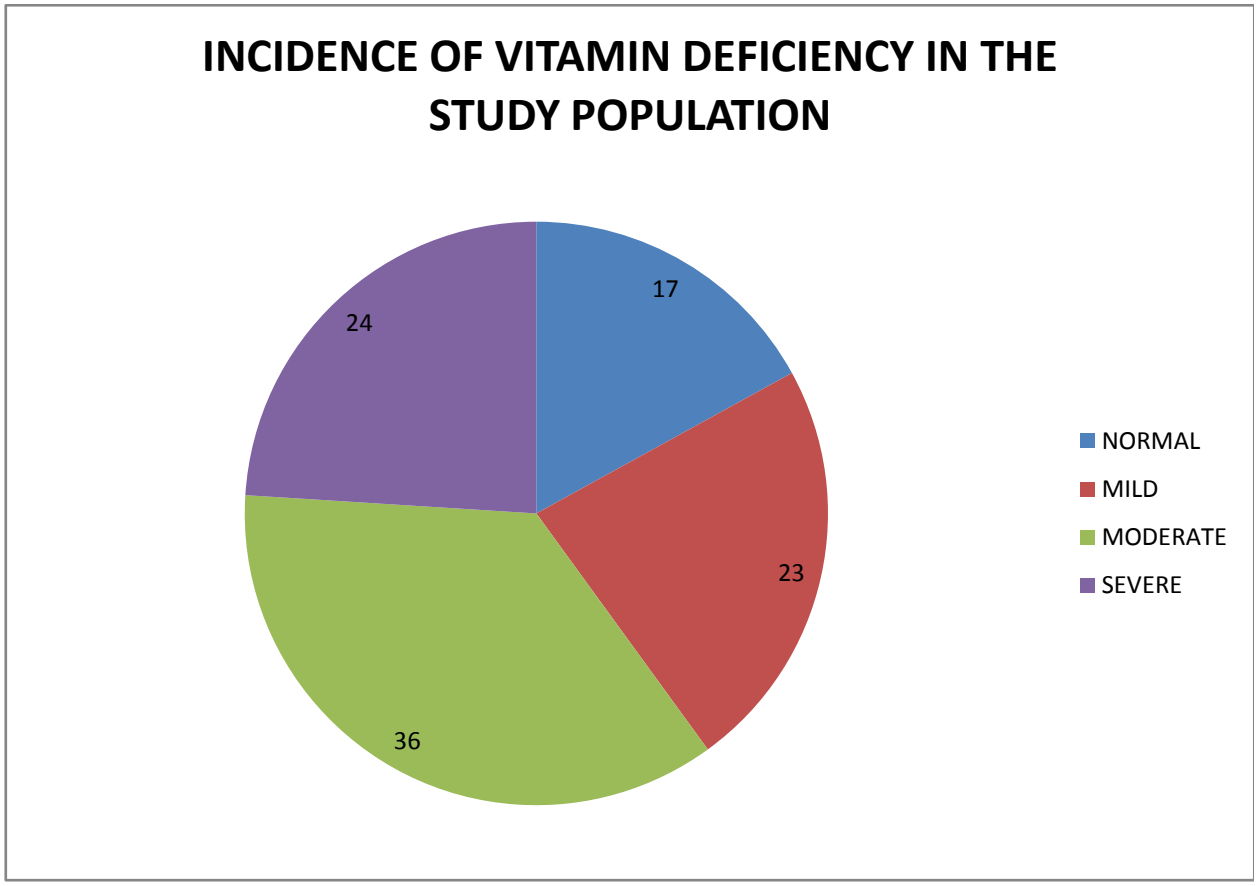
19 patients in the critical limb ischemia group (38%) and 22 patients in the claudicant group (44%) were hypertensive. The incidence of hypertension as a risk factor of arterial disease was similar in both the groups of the arterial disease.

Graph9



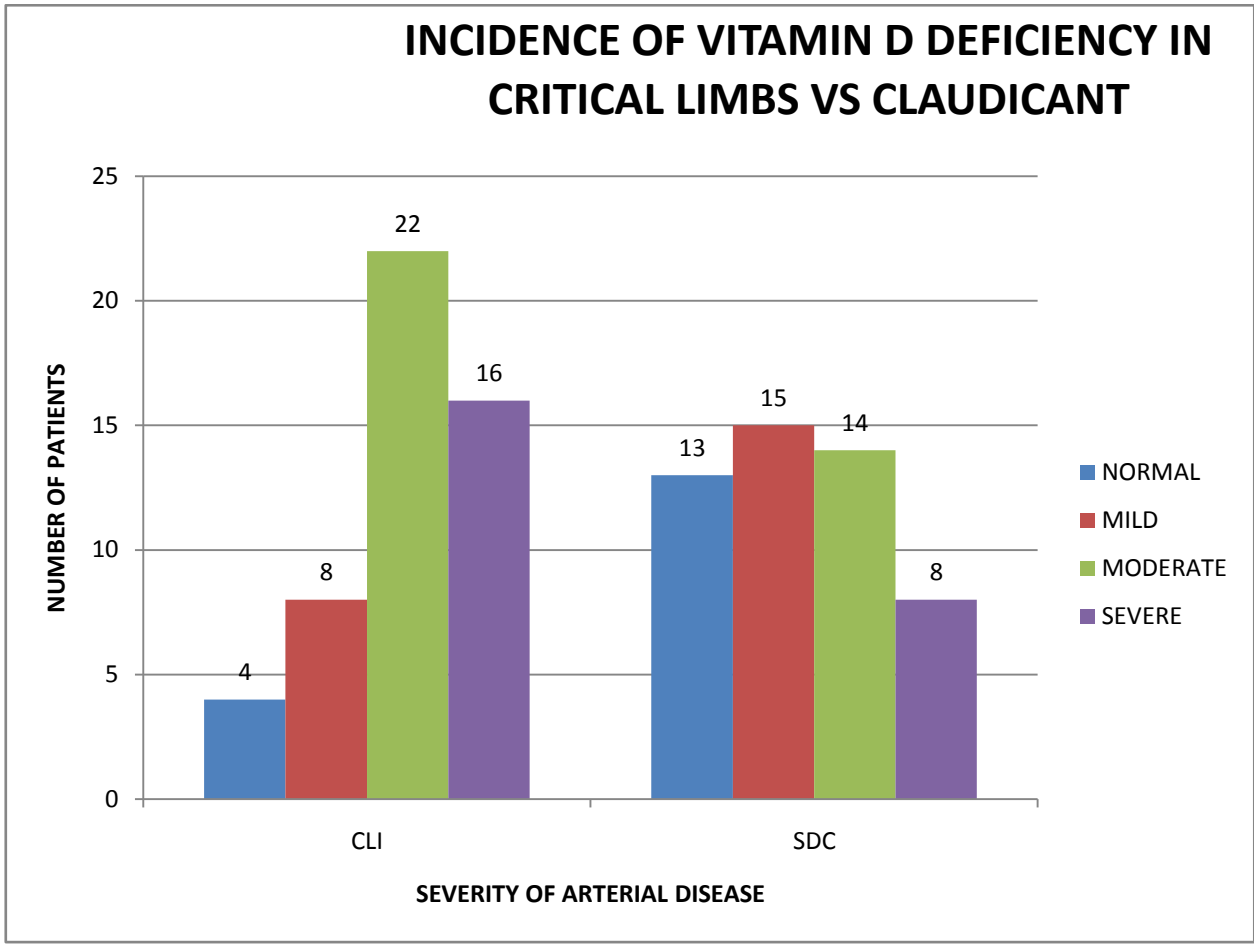
16 patients in the critical limb ischemia were diabetic (32%) while 13 patients in the claudicant group were diabetic (26%). The incidence of diabetes in both the arterial groups were similar and the difference was not statistically significant.

Graph10



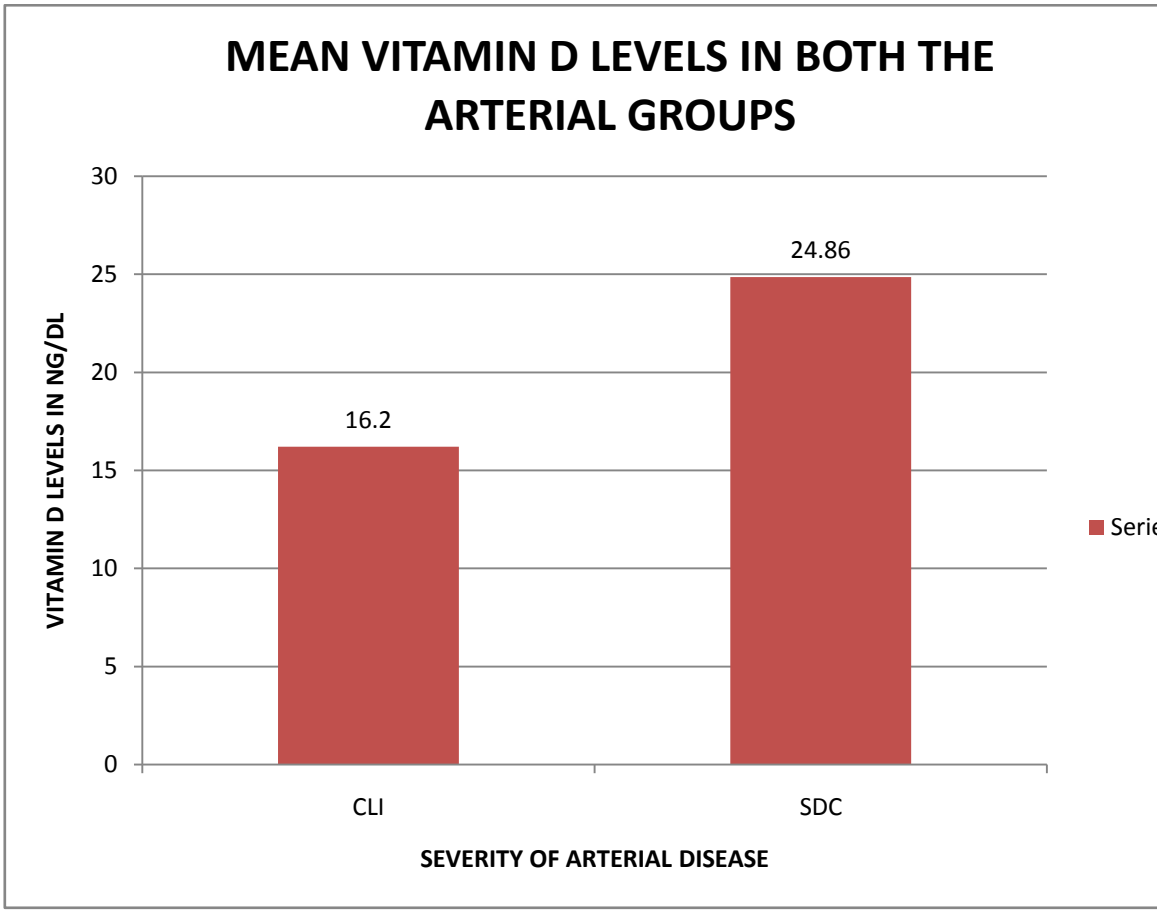
There were 17 patients who had normal vitamin D levels, 23 patients who had mild deficiency, 36 patients who had moderate vitaminD deficiency and 24 patients who had severe vitamin D deficiency. In the study population the incidence of vitamin D deficiency was 83 % which was comparable to other studies done in South Indian population.

Graph11



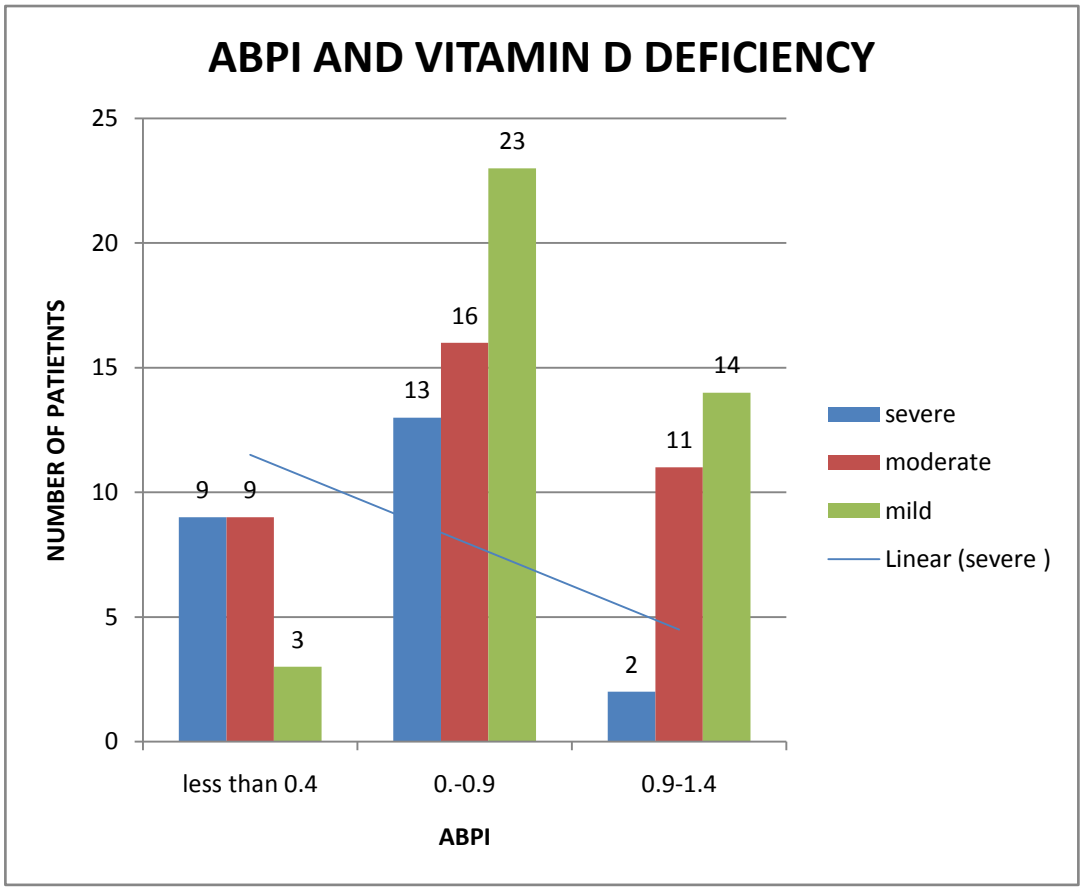
The incidence of severe vitamin d deficiency in the critical limb ischemia group was 32.7% while in the claudicant group was 14%. The difference in the vitamin D values were statistically significant (p value –0.004). Moreover it is noticed that as the severity of the disease increases, the deficiency levels worsen. This is better explained in the next table.

Graph12



The mean vitamin D value in the critical limb ischemia group was 16.2 ng/ml while the mean in the claudicant group was 24.86 ng/ml. The difference in the vitamin D levels were statistically significant (p value – 0.001)

Graph 13

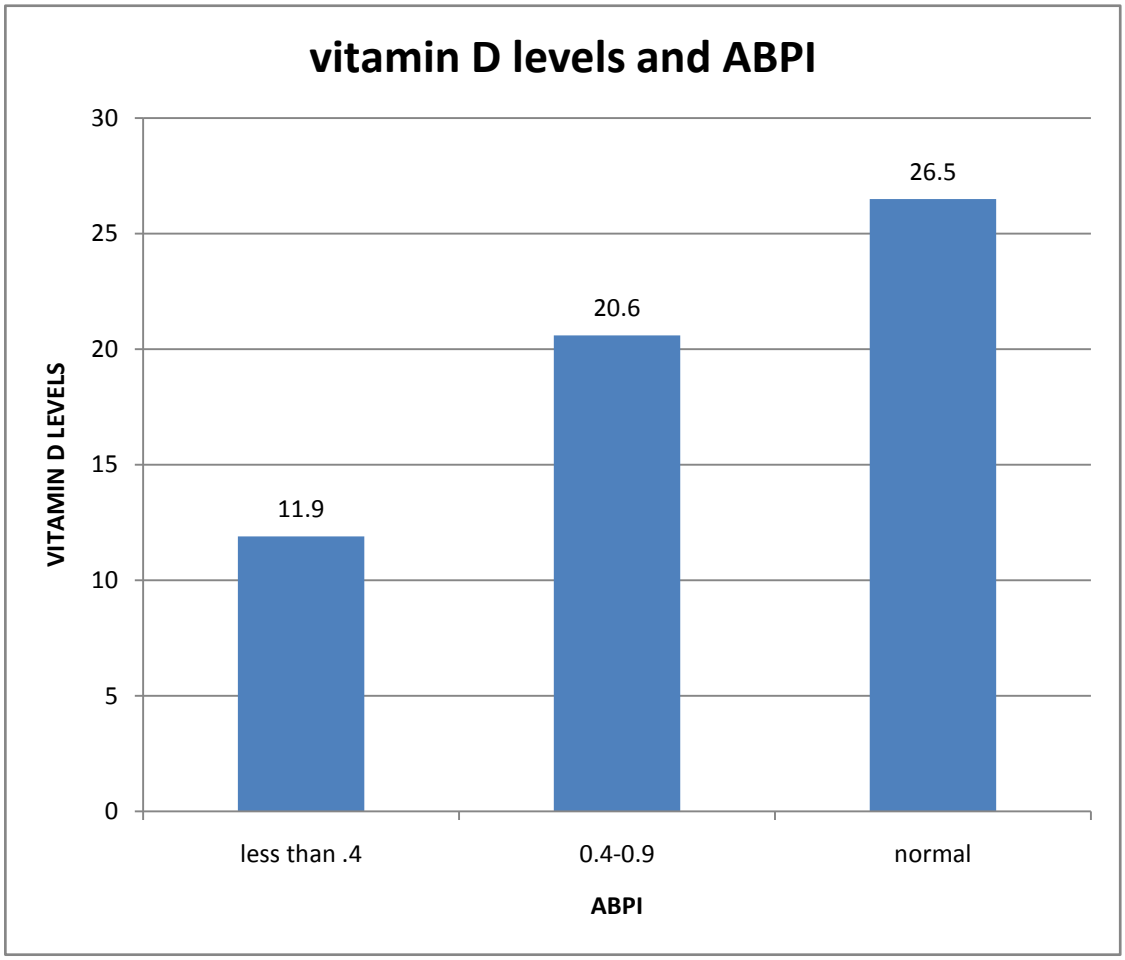


In our study we have considered ABPI as a marker for atherosclerotic disease. ABPI has been shown to predict overall survival independent of other conventional cardiovascular risk factors. Decreasing ABPI has been associated with a increasing hazard ratio in the Framingham study.

In the above graph it is evident that patients with lesser ABPI had lesser Vitamin D levels (linear trend). The vitamin D levels were higher in the patients with claudication only.

In the above graph only 21 patients had documented ABPI less than 0.4, the remaining patients had evidence of tissue loss and ulcer with a subcritical ABPI. This could be due to vessel wall calcification seen in diabetes and chronic kidney disease. The correlation between the low vitamin D levels and low ABPI was statistically significant (p value – 0.02) and it is better explained in the graph below.

Graph14



The mean vitamin D level in the (< 0.4) ABPI group was 11.9 ng /dl, in the ABPI group(0.4 to 0.9) was 20.6 ng/dl and the mean in the normal group was 26.5 ng/dl. Thus an upward trend is noticed.

DISCUSSION

This study seems to show a strong association between the vitamin D status and severity of PAOD. This seems to be independent of the other cardiovascular risk factors. The prevalence of vitamin D deficiency in these patients seems to be very high. This is more than many of the western reports inspite of the sunny nature of our climate. Moreover as the severity of the disease increases the vitamin D status also worsens. This is probably due to dietary and poor ambulatory status. The question we would like to know is if the inverse is true. Does low levels of vitamin D actually worsen the disease? Vitamin D has been identified as an risk factor for cardiovascular events. Moreover we also noticed that deteriorating ABPI are associated with low vitamin D status. There are very few studies that have mentioned ABPI status in patients with vitamin D deficiency. We also found that the association between the low vitamin D status and severity of the PAOD was independent of hypertension, diabetes and dyslipidemia. This was in contrast to other similar studies.

This study seems to suggest that low vitamin D status seems to have an independent effect on atherosclerotic arteries resulting in disease progression. The vitamin D receptors which are ubiquitous may play an important role by interacting with the endothelial cells and vascular smooth muscles. The low levels of vitamin D seem to cause inflammation and vascular calcification.

There are several limitations to this study. The question of whether this association is influenced by other confounding factors like colour of skin, ethnicity, poor living conditions, socioeconomic scores, diet and drug interaction has not been studied. We have not been able to follow up these patients due to various reasons.

In conclusion primary and secondary prevention strategies should also include vitamin D status .The question of vitamin D supplementation in these patients needs further randomized trials. Moreover there needs to be international standardizing of the vitamin D levels. Low vitamin D levels is a risk factor for the severity of the PAOD .

CONCLUSION

Now that we are sure that vitamin D deficiency plays an important role in atherosclerosis and thus can be considered a modifiable risk factor for cardiovascular disease. The next reasonable step will be to assess the burden of this disease in patients with PAOD. Moreover since there are two kinds of these patients within the same subset (claudicants and critical limb ischemia patients) we propose to see if there is a significant difference in the vitamin levels within these two subsets. This could lay the ground work for future interventional studies that could identify if primary or secondary prevention of PAOD is possible with supplements of vitamin D. Moreover we might be able to answer the question of whether vitamin D supplementation to claudicants gives them an additional beneficial effect.

However a major drawback for any clinical strategy is patients and physician compliance despite the various guidelines. Poor compliance is more often an unrecognized risk factor for cardiac events. Many patients in developing countries cannot afford treatment or even the diagnostic test. The drugs like statins arte very expensive for the common man if there are no government subsidies. The number of daily pills, the overlap of chronic diseases, development of adverse effects also result in poor compliance. Hence any strategy for risk

factor modification should also take into consideration the compliance levels of the community.

The proper selection of long acting drugs and to decrease the frequency of doses will improve the compliance. Vitamin D given as once a week strategy will help in improving the compliance. Increased awareness of these possible new risk factors, introduction of educational programmes , active participation of the government and nongovernmental organization will improve the comprehensive care of the patients.

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Study data :

NAME	VIT D		ABPI	LIPIDS	HTN	DM	SMOKER	GENDER	AGE
MD SALIM	13.85	SDC	0.62	y	NO	Y	YES	M	59
SUNIL DAS	21.17	SDC	0.62	n	NO	N	YES	M	57
SUKUMAR BAG	17.17	SDC	0.5	n	NO	N	YES	M	55
BIPUL MONDAL	26.09	SDC	1	n	NO	N	YES	M	43

PARTHIBAN P.	28.21	SDC	1	n	NO	N	YES	M	59
CHITTARANJAN GHOSH	31.2	SDC	0.72	n	NO	N	YES	M	55
CHANDRAKALA.T	14.15	SDC	1	n	NO	N	NO	F	28
VALLIYAMMAL	9.45	SDC	0.9	n	NO	N	NO	F	47
BISWANATH HALDER	22.05	SDC	0.73	n	NO	N	YES	M	59
GOUR CHANDRA SARKAR	25.54	SDC	0.88	n	NO	Y	YES	M	65
MAMANI BARMAN	14.41	SDC	0.9	n	NO	N	NO	F	46
SURESH RAO.K.	10.28	SDC	0.9	n	NO	N	NO	M	37
JOHNY K.C.	25.79	SDC	0.62	n	NO	N	YES	M	62
SELVARAJ R	10.31	SDC	0.38	n	NO	N	NO	M	55
SAMBAU PITCHAIAH	25.63	SDC	0.87	n	NO	N	YES	M	56
NUR MAHAMMAD MIYA	29.61	SDC	1	n	NO	N	YES	M	60
AJIT PRADHAN	51.01	SDC	1	n	NO	N	YES	M	26
AZZEZUNISSA BEGUM	31.36	SDC	1.31	n	NO	N	NO	F	75
DIPAK MAGHI	37.13	SDC	0.55	n	NO	N	NO	M	37
ANIL CHANDRA RAY	53.3	SDC	0.45	n	NO	N	YES	M	50
GOWRI.G	28.17	SDC	1	n	NO	N	NO	F	60
CHITRA P.	22.37	SDC	1	n	NO	N	NO	F	35
SUKLAL PRAMANICK	5.46	SDC	0.74	n	NO	N	YES	M	48
PERUMAL	24.18	SDC	0.5	n	NO	N	YES	M	21
BHAGOT MUKHIM	35.3	SDC	1	n	NO	N	YES	M	58
CHANDRASEKARAN.K.	53.58	SDC	0.78	n	NO	Y	YES	M	67
SUDHANGSHU RANJAN DAS	8.75	SDC	0.79	n	NO	Y	YES	M	55
GOVINDARAJ.K	25.86	SDC	0.55	n	NO	Y	YES	M	78
MOHAMMED GOLAM HOSSAIN	11.82	SDC	1	y	YES	Y	YES	M	60
MD. SALIM	13.53	SDC	0.71	y	YES	Y	YES	M	55
RANAJIT CHAKROBORTY	7.74	SDC	0.85	y	YES	Y	YES	M	69
BABU RAO.A	12.15	SDC	0.17	y	YES	Y	YES	M	56
SATYA RANJAN DAS	30.49	SDC	1	n	YES	N	YES	M	64
MAYILVAGANAM	23.86	SDC	2.14	n	YES	Y	YES	M	76
SHAHEB PARVAZ KHAN	6.19	SDC	0.58	n	YES	N	YES	M	61
SWAPAN KUMAR KUNDU	23.07	SDC	0.8	n	YES	N	YES	M	67
CHELLAMMAL.D.A	6.3	SDC	0.91	n	YES	N	NO	F	72
NATA RAJAN	18.52	SDC	0.63	n	YES	N	YES	M	68
SUDIR KUMAR	70	SDC	0.46	n	YES	N	YES	M	65
RAMALINGAM P.	19.72	SDC	1.25	n	YES	N	NO	M	63
MADHUSUDAN DAS	33.89	SDC	1.08	n	YES	N	YES	M	58
HRISHIKESH DAS	6.4	SDC	0.38	n	YES	Y	YES	M	66
NARAYANA.K.	18.69	SDC	0.85	n	YES	N	YES	M	58
PALLE SADANANDA SUVISESHAM	< 3.00	SDC	0.38	n	YES	N	YES	M	67
RAMACHANDRAN	26.34	SDC	0.15	n	YES	N	NO	M	58

RAVINDRA KUMAR JOEL	> 70.00	SDC	1	n	YES	N	YES	M	61
ZAKERA BEGUM	15.44	SDC	0.4	n	YES	Y	NO	F	55
KARTICK CHANDRA MONDAL	39.28	SDC	0.65	n	YES	N	YES	M	68
AMALENDU CHAKRABORTY	16.76	SDC	1	n	YES	N	YES	M	69
GEORGE P.A.	53.43	SDC	0.58	n	YES	Y	YES	M	58
LAXMI PAUL	5.67	CLI	0.35	n	NO	N	NO	F	42
JYOTI RONI DEBNATH	6.68	CLI	0	n	NO	N	NO	F	47
SAKINA BEE.S.D.	6.68	CLI	0.51	n	NO	N	NO	F	64
KARPURA PANDA	7.01	CLI	0	n	NO	N	NO	F	68
TANDRA BASU	22.4	CLI	0.5	n	NO	N	NO	F	56
DEVAKI SHARMA	23.58	CLI	0.6	n	NO	N	NO	F	74
DURGABAI	3.04	CLI	0.14	n	YES	N	NO	F	80
CHENGAMMA	14.62	CLI	0.58	n	YES	N	NO	F	83
RIZWANA	5.06	CLI	0.55	n	NO	Y	NO	F	41
KUPPAMMAL	12	CLI	1	n	YES	Y	NO	F	60
LAKSHMI M.	4.35	CLI	0.7	n	YES	Y	NO	F	42
KANNABIRAN	34.6	CLI	0.75	n	NO	N	NO	M	51
VICTOR M.	36.03	CLI	0.53	n	NO	N	NO	M	78
RAGHAVENDRA M S	< 3.00	CLI	0.4	n	NO	N	NO	M	72
CHARLES P.	7.86	CLI	1.21	n	YES	N	NO	M	84
SENTU BASAK	10.71	CLI	0.72	n	NO	Y	NO	M	49
BALASUBARAYADU	8.59	CLI	0.2	n	YES	Y	NO	M	71
PALANIAPPAN R.	3.25	CLI	0.57	n	NO	N	YES	M	56
MD BASHIR ULLAH	7.46	CLI	0.64	n	NO	N	YES	M	45
NARENDRA MAROTI	8.41	CLI	0.75	n	NO	N	YES	M	61
GOPAL CHANDRA MAZUMDER	11.27	CLI	0.47	n	NO	N	YES	M	63
UTPAL KUMAR DEY	13.33	CLI	0.45	n	NO	N	YES	M	60
MUNIYAPPA	13.37	CLI	0.22	n	NO	N	YES	M	27
SIVAKUMAR K.	15.21	CLI	0.33	n	NO	N	YES	M	48
SIDDHESWAR MONDAL	15.52	CLI	1.37	n	NO	N	YES	M	59
TULA RAM	16.04	CLI	0.3	n	NO	N	YES	M	67
GUNTI VENKATACHALAM	18.57	CLI	1.09	n	NO	N	YES	M	75
DEVARAJ G	19.23	CLI	0.62	n	NO	N	YES	M	85
KASI	19.75	CLI	0.95	n	NO	N	YES	M	41
VENKATESAN	25.76	CLI	0.85	n	NO	N	YES	M	45
ARUNDAS	26.57	CLI	0.37	n	NO	N	YES	M	53
LAL MOHAN BAURI	29.78	CLI	0.58	n	NO	N	YES	M	39
VIJAYAN GOPAL	4.08	CLI	0.14	n	YES	N	YES	M	61
GOPAL REDDY	16.09	CLI	0.92	n	YES	N	YES	M	80
DURGA BHATTACHARJEE	17.32	CLI	0.58	n	YES	N	YES	M	62
NARAYANASAMY R.	17.74	CLI	0.39	n	YES	N	YES	M	84

GANESH BHAKAT	21.33	CLI	0.78	n	YES	N	YES	M	44
ANANYA DEY	59.61	CLI	1.18	n	YES	N	YES	M	58
SYD NOUSHAD BASHA	7.59	CLI	0.8	n	NO	Y	YES	M	51
DURAISWAMY R.	12.35	CLI	0.5	n	NO	Y	YES	M	66
LAKSHMI KANTHAIAH	15.53	CLI	0.7	n	NO	Y	YES	M	70
RADHASHYAM PAL	15.56	CLI	0.29	n	NO	Y	YES	M	65
DAKSHINAMOORTHY	4.29	CLI	0.73	n	YES	Y	YES	M	37
PAWAN PARIHAR	16.83	CLI	1.18	n	YES	Y	YES	M	58
KASI VISWESWAR RAO K.	16.89	CLI	1.92	n	YES	Y	YES	M	68
SIVALINGAM M	18	CLI	0.56	n	YES	Y	YES	M	68
ABDUL RAHAMAN	21.8	CLI	0.13	n	YES	Y	YES	M	60
MRITYUNJOY SARDAR	54.66	CLI	1.23	n	YES	Y	YES	M	65
BISHNU PRASAD KAR	24.92	CLI	0.68	y	YES	N	YES	M	71
DILIP DUTTA	12.01	CLI	0	y	NO	Y	YES	M	58

Abbreviations

SDC- short distance claudicants

CLI- Critical limb ischemia